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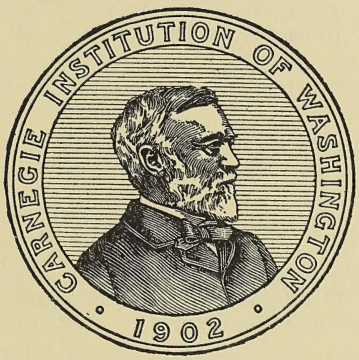
PAPERS FROM TORTUGAS LABORATORY OF CARNEGIE INSTITUTION OF WASHINGTON

Vol. XXIX



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PAPERS FROM TORTUGAS LABORATORY, VOL. XXIX

I

REGENERATION IN THE CIRRATULID CIRRINERIS

By Raymond G. Stone

Zoological Laboratory, University of Missouri

[Issued November 30, 1935.]

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REGENERATION IN THE CIRRATULID CIRRINERIS

INTRODUCTION [1]

There has been considerable study of polychæte regeneration within the last few years, but the observations are not as extensive as those among the oligochætes. The external changes and histology of regeneration are well known in certain groups of polychætes in which the amount and origin of the new tissues are variable. It has been shown in some cases (*cf.* Faulkner, 1932) that mesodermal reserve cells are present and only become active during regeneration. However, their behavior and appearance are different from the neoblasts of oligochætes. In other polychætes there is no reserve material and the new structures are derived from the old tissues.

Since no account of Cirratulid regeneration is found in the work of previous authors, this investigation of *Cirrineris* was undertaken to determine the extent of segment replacement and the source of the new tissues.

Material was secured at the Tortugas Laboratory of the Carnegie Institution during the summer of 1932. The histological preparations were made at the University of Missouri and my thanks are due this institution and Professor W. C. Curtis of the Zoology Department for the help and materials furnished.

MATERIALS AND METHODS

The specimens were obtained from the Fort Jefferson moat on Garden Key near the Tortugas Laboratory. They are found in clumps of *Valonia* growing along the moat wall and are easily collected when these masses are broken apart.

Cirrineris collected during June and July 1932 were composed of approximately 115 segments. However, the worms may not have reached the maximum adult size since none of them contained mature gonads. The body consists of a head region of 3 to 4 segments free from tentacles and setæ. The remainder of the worm is about equally divided into the tentacle-bearing region and the terminal abdominal segments. No specialized branchial filaments were found on any of the worms.

The head region plus several of the tentacle-bearing segments posterior to it was severed for anterior regeneration and approximately two-thirds

[1]This investigation of *Cirrineris* regeneration is intended to be preliminary to a study of the effects of radium radiations on regeneration in this annelid. It is in continuation of previous work at the University of Missouri supported in part by the Committee on Radiation of the National Research Council.

of the abdominal segments were removed at the posterior end. At this level of the body the segments are larger than near the posterior end and the new tissues are more easily distinguished from the old body region.

Regenerating worms were kept in large crystallizing dishes of sea-water in the laboratory and the amount of new tissue was checked at two-day intervals. Specimens at various stages of regeneration were killed in Heidenhain's trichloracetic acid mixture. Material for histological study was sectioned longitudinally, 5 to 8μ, and stained with Delafield's hematoxylin and iron hematoxylin with Orange G.

OBSERVATIONS

EXTERNAL CHANGES

Anterior Regeneration

Anterior segments are readily replaced by *Cirrineris*. After removal of six to eight segments, the intestinal epithelium is everted and spreads over the wound surface to meet the cut edges of the body wall. The wound is thus closed quickly with little loss of body fluids. This method of wound closure is also found in other polychætes (*cf.* Probst, 1931; Sayles, 1932; Stone, 1933*a*). Two days after the operation, the region is slightly constricted and the everted intestine lining is no longer seen. During the third day a blunt elevation of new tissue arises at the severed end. This knob of new tissue increases in size and by the fourth day the mouth opening and prostomial enlargement are present. After eight days' regeneration, the new region has increased greatly in length, apparently by addition of new material adjacent to the old body segments. At the same time the external constrictions of four to six new segments appear in this region and the new tentacles grow out from their dorso-lateral surfaces. After ten days' regeneration, the head region and six to seven new segments posterior to it are present. No more segments are added after this, and by the eighteenth day the new region has reached adult size.

Posterior Regeneration

In the posterior half of the body, *Cirrineris* regenerates at practically the same rate from any level. In these experiments, however, only the twenty to thirty terminal segments were removed. The wound is closed by extension of the everted intestine lining over the cut surface in a manner comparable with closure at the anterior end. Constriction of the wound region and retraction of the everted gut lining occur the second day after operation. A small knob of new tissue appears about the anal opening the following day. Regeneration is evidently more rapid ventrally, for this region soon becomes longer. After five days, the regenerating region extends approximately the length of two old body segments and is about half their diameter. The higher rate of regeneration ventrally pushes this region

upward around the tip of the new tissue until the anus opens dorsally (figs. 9 and 10). After several days, the anal opening is again shifted to a terminal position. The external annulations of several new segments appear near the base of the regenerating region by the sixth day. The number of new somites increases rapidly until an average of twenty has been formed by the sixteenth day, when all segments except a few near the tip of the worm have reached adult size.

INTERNAL CHANGES

Anterior Regeneration

Sections of worms killed fifteen minutes after severing the anterior segments show the intestinal epithelium already everted and spread over most of the wound surface. One hour later this layer extends to the epidermis and the wound is completely covered. The cut edges of the two epithelial layers usually unite by the end of the first day. In some cases this may be delayed until the following day by injured muscle cells projecting between the edges of the layers. During the second day, the severed end is constricted by contraction of the body-wall muscles and the everted gut lining is withdrawn. The edges of the epidermis are thus brought together over the wound, where they usually unite. There is no aggregation of injured muscle cells and leucocytes to form a cicatrix in the wound region as reported by Nusbaum (1905, 1908) in *Nereis diversicolor* and other polychætes.

The appearance of the epidermal layer at the severed anterior end remains the same until the third day, when the cells and nuclei become much enlarged. This is most evident ventrally anterior to the cut end of the nerve cord, but no mitoses were seen in this region. These epidermal cells have moved anteriorly around the exposed end of the nerve cord (fig. 2) as the everted gut epithelium was withdrawn. By the fourth day numerous mitoses have appeared in the epithelial layers and a knob of new tissue extends from the severed end. The projecting lobe of the prostomium is present above the mouth opening (fig. 3). On account of the low magnification of the drawings, mitotic figures in the epithelial layers are not shown in detail. They are represented as larger and more heavily stippled cells. Epidermal proliferation is most active in the antero-dorsal region of the prostomium, where cells are pushing inward to form the new cephalic ganglia. At the same time the thickened ventral epidermis forms the new nerve cord. Nerve fibers push into the new region from the old nerve cord (figs. 3 and 4) and the epidermal cells differentiate into the new ganglion cells and ganglia. Iwanow (1908) described a similar process in *Spirographis spallanzanii*. During the fifth and sixth days, epidermal proliferation continues and the new region becomes much longer. New material is also added to the ventral and cephalic ganglia, which are now united by

connective fibers. The brain consists of an irregular mass covered with ganglion cells on its dorsal and lateral surfaces. The connectives join it anteriorly.

Small pits lined with elongated ciliated cells appear in the lateral walls of the prostomium during the fifth day. They are similar to the prostomial sensory areas described by Iwanow (1908) in *Spirographis*. There is no further change, except increased size, in structures derived from the epidermis while the regenerated segments are attaining adult size. The everted intestine lining is withdrawn during the second day and the edges of the epidermis push forward and unite over the wound. The severed gut heals and is withdrawn a short distance from the terminal epidermis (fig. 2). The end of the gut then elongates to meet the small stomodæal invagination with which it fuses, and by the fourth day the intestinal lumen again opens to the outside (fig. 3). At this time numerous mitoses are present in the enlarged epithelial cells of the pharynx, and this proliferation continues until the new structures have reached adult size.

The longitudinal muscles of the body wall and intestine are replaced from mesodermal material. The fibrillar portions of the injured muscle cells near the wound degenerate by the third day and their nuclei with the remainder of the cytoplasm are liberated into the body cavity. At the same time the peritoneal cells in the terminal segment become enlarged and move toward the wound. Here, together with the nuclei of the muscle cells and connective tissue cells from the body wall, they form a syncytial mass of cells (fig. 2) which fills the body cavity in the regenerating region. Similar behavior of these cells has been reported during regeneration in Syllids by Okada (1929), in *Diopatra amboinesis* by Pflugfelder (1929), and in *Aricia fœtida* by Probst (1931). With the peritoneal cells in *Cirrineris*, this activity never extends beyond the first septum posterior to the wound. It is especially noticeable along the nerve cord. From the third to the sixth day, the syncytial mass appears as a layer at the base of the epidermis where the longitudinal muscles of the body wall are being formed. At the same time a thicker layer is laid down around the new pharynx to form the much heavier musculature about this organ. The syncytial cells extending throughout the body cavity in the regenerating region (fig. 3) form the first new septa ventro-laterally. By the ninth day the muscle layers of the body wall and pharynx are clearly differentiated from the regeneration cells and the circular body-wall muscles are beginning to appear. Nusbaum (1908) in *Nereis diversicolor* reported participation of the basal epidermal cells in formation of these muscles, but their source was not determined in *Cirrineris*. The movement of peritoneal cells from the old body region ceases after the sixth day. At this time the new mesodermal structures have been laid down in the manner indicated, and the growth of these organs during the later stages of regeneration occurs by proliferation within them. The syncytial cell mass decreases in size and disappears by the twelfth day.

Irregular blood lacunæ extend from the severed ends of the old blood-vessels into the syncytial cell mass. These lacunæ are most numerous in the prostomial region below the newly formed cephalic ganglia (fig. 4) and the syncytial cells surrounding them differentiate into the blood-vessel walls. By the ninth day the large dorsal and ventral blood-vessels are formed and only the smaller connecting vessels at the anterior end remain to be completed.

New tentacles appear as dorsal epidermal evaginations near the base of the regenerating region (fig. 5). Regeneration cells push into them and form a layer of longitudinal muscles at the base of the epidermis. The walls of the paired blood-vessels within the tentacles are also formed from syncytial cells, many of which remain after regeneration is complete. The bundles of nerve fibers which extend along opposite sides of the tentacle between the base of the epidermis and the longitudinal muscle layer are formed by epidermal proliferation.

A heart-body similar to that described by Dryssen (1912) in *Audouinia filigera* is present in *Cirrineris*. This organ lies within the dorsal blood-vessel and extends for 40 to 50 segments posterior to the head region. It increases in size between the septa, and the successive enlargements are connected by a small strand of cells through the blood-vessel piercing the septum (figs. 4 and 5). The heart-body is formed from syncytial cells which lie in the dorsal part of the regenerating region. The new septa separate masses of these cells which differentiate into heart-body tissue and the surrounding blood-vessel walls. This origin is similar to development of the heart-body in *Polymnia nebulosa* described by Picton (1898).

Posterior Regeneration

Wound closure at the posterior end follows essentially the same course as at the anterior end. The wound is almost completely covered by the everted gut lining within a few hours (fig. 6); the mass of injured muscle cells in the terminal segment may also aid in closing the body cavity at the junction of the epithelial layers. During the second day the edges of the epithelial layers unite and the gut lining is withdrawn to its normal position.

Epidermal cells in the ventral region of the terminal segment become enlarged during the second day and mitoses appear in this region on the third day. Proliferation also begins in the dorsal epidermis on the fourth day. This activity continues throughout the period of regeneration and always is most pronounced in the ventral region (figs. 8 and 9). Mitoses are represented by larger, more heavily stippled cells in the figures. The nuclei of the proliferating epidermal cells are enlarged and contain prominent nucleoli; Sayles (1927) described similar behavior in regenerating *Lumbriculus*.

Two days after severing the worm, the end of the nerve cord lies against the ventral body wall adjacent to the enlarged epidermal cells (fig. 11). It

never extends through the ventral epidermis as at the anterior end (*cf.* figs. 1 and 2). Beginning with the third day, nerve fibers extend posteriorly from the cut end of the nerve cord along the median line of the ventral epidermis (fig. 8). The basal cells of the proliferating epidermis push in about these nerve fibers and form the new ganglion cells and ganglia. The old ganglion cells do not participate in regeneration except by extension of their fibers. Similar observations have been reported by Pflugfelder (1929) in *Diopatra* and by Schultz (1899) in several other polychætes. This process of nerve-cell differentiation continues as long as new segments are being regenerated. The inter-segmental connectives later become separated from the epidermis by circular muscle fibers in the fully differentiated segments, but the ganglia retain their connections with the basal epidermal cells (figs. 8, 9 and 10).

The cells of the intestinal epithelium enlarge by the third day and begin to divide. This proliferation continues throughout regeneration (figs. 8, 9 and 10) and is the source of material for the intestinal lining in the new segments.

Mesodermal tissues at the posterior end regenerate from the old mesoderm. As previously described in anterior regeneration, only the cells in the terminal segment are affected and they are most active in the ventral region about the nerve cord. The nuclei and cytoplasm of the injured longitudinal muscle cells and the connective tissue elements join the mass of enlarged peritoneal cells (figs. 7 and 8); all the new mesodermal structures are formed from this material. This corresponds to the observations of Okada (1929) that all mesodermal elements were "mesenchymatized" at first as the source of new material in Syllid regeneration. New septa begin to differentiate from the mass of dividing syncytial cells (fig. 9) by the fourth day and the new body wall and intestinal musculature is formed from the same source. Lacunæ extend from the old blood-vessels into the regenerating region and the surrounding cells differentiate into the walls of the new blood-vessels. Numerous mitoses are present in the ventral mass of syncytial cells (figs. 8 and 9) while they are carried posteriorly by addition of new segments. The nuclei of these cells are large and frequently contain prominent nucleoli (fig. 11), while the amount of cytoplasm in the syncytial mass is relatively small. Many large cells remain upon the septa and inner surface of the body-wall musculature in newly regenerated segments. These cells decrease in size and form the new peritoneum.

DISCUSSION

Reserve cells are often concerned in the regeneration of new mesodermal tissues in polychætes; yet the degree of participation of these cells varies in different worms. Only the reserve cells are capable of active proliferation in regenerating *Filograna implexa* and *Chætopterus variopedatus* according to Faulkner (1930, 1932), and they form all the new tissues. Probst (1931)

described a similar origin of new tissues in *Aricia fœtida*. In contrast to this condition, Stolte (1929) finds that *Polyophthalmus pictus* Duj. is unable to regenerate. Mesodermal reserve cells are present; but they are active only in the tissue changes concerned with growth of the gonads. In explanation of this condition in *Polyophthalmus*, it is possible that the injured tissues are unable to activate the reserve cells. This would be similar to the situation in *Tubifex* (Stone, 1933*b*) in which reserve cells are present in anterior regions and participate in regeneration posterior to them, but are not activated by an anterior wound.

Extensive regeneration is possible from the old mesodermal elements in the absence of reserve cells. This method of replacement was described in the Syllidæ by Okada (1929) and in *Owenia fusiformis* by Probst (1932). This is also the method of regeneration in *Cirrineris*, in which the amount of material derived from the old tissues is limited, since only the tissues in the terminal segment adjacent to the wound are affected. The old mesodermal tissues undergo a mesenchymatization which involves a degeneration of the formed elements in highly differentiated cells.

In *Cirrineris* the muscle-cell nuclei and cytoplasm with the connective tissue cells, freed by degeneration of contractile elements of the musculature (*cf.* figs. 6, 7), are added to the syncytial material, but the peritoneal cells are the chief source of new mesoderm. There is no visible differentiation in the cytoplasm of these peritoneal cells comparable to that found in other tissues. The cells are flattened as a lining layer of the body cavity and are smaller and more numerous ventrally; their transformation into active regeneration cells apparently involves no change other than detachment from the body wall and movement toward the wound.

New tissues may also arise from reserve cells with the addition of varying amounts of material from the old mesodermal structures, as Pflugfelder (1929) described in *Diopatra amboinensis*. In agreement with other authors, he finds that the differentiated cytoplasm of the old mesodermal cells degenerates before the cell nuclei participate in regeneration.

There are also a few worms without reserve cells, in which the old mesoderm does not participate in regeneration. It has been shown in *Nereis diversicolor* (Nusbaum, 1908) and in *Euratella* (Stone, 1933*a*) that all the new tissues are derived from epidermal material. Cells from this source push into the body cavity and form the new mesodermal structures. With exception of worms in which all the new tissues arise from reserve cells, the epithelial layers and nervous tissues in other groups regenerate by epithelial proliferation.

The problem of how the mesodermal cells are activated in *Cirrineris* should be less complex than in other polychætes, in which regeneration cells become active several segments from the wound and move toward the injury. Mesoderm cells in the terminal segment of *Cirrineris* may respond directly to the stimulus of the injured tissues on account of their proximity

to the wound; or they may react to dilution of the body fluids by water, as Sayles (1931) found in the epithelia of *Lumbriculus*. There is no direct evidence for either of these possibilities, although the inactivity of the peritoneal cells beyond the adjacent septum would indicate a localized stimulus. The theory of "humoral" activation proposed by Stolte (1933) in *Dero* could also be applied in the case of *Cirrineris*. The terminal segment is filled with fluid from the severed blood-vessels before the peritoneal cells become active, and the large blood-filled lacunæ which extend throughout the regenerating region would provide suitable conditions for such activation in the proliferation and differentiation of new material. No blood is liberated into the body cavity beyond the adjacent septum, and here the peritoneal cells are inactive. If this factor is effective in *Cirrineris*, it is probable as Stolte suggests, that activation is dependent to some degree upon the increased concentration of nutrients supplied by the blood and cœlomic fluid in the congested regenerating region.

The ventral epidermis in the terminal posterior segment of *Cirrineris* is the first area to begin active proliferation of new material. This is in agreement with observations on regenerating oligochætes. Krecker (1923) and Stolte (1933) believe that the ventral mass of regeneration cells must have a stimulating effect which brings about the embryonalization of the adjacent old epidermal cells. On this basis we can understand the apparent correlation between epidermal proliferation in *Cirrineris*, which begins in the ventral region and spreads laterally and dorsally, and the similar movement of syncytial material from the ventral mass.

Cirrineris regeneration can be used in determining the differential effects of radiations on the different sources of replacement material. The differentiated cells here, as has been shown in other forms, vary in their susceptibility to radiations and some of them might be unable to participate in regeneration after suitable treatment. The relative importance of the various tissues as sources of new material could be determined by this method. Radiated tissues in *Tubifex* (Stone, 1933*b*) are able to participate in wound healing, and we would expect activity of similar tissues in *Cirrineris*. After varying exposures to radiation, mesodermal regeneration would proceed as far as permitted by the limited supply of replacement material.

It is hoped that the opportunity for application of the radiation technique to *Cirrineris* regeneration will soon arise.

SUMMARY

1. A head region and six to seven segments posterior to it are regenerated when more than this number are removed.

2. Posterior regeneration is rapid and complete; the approximate number of segments removed is replaced.

3. Wound closure is effected in the same manner at both ends of *Cirrineris*. The edges of the everted intestine unite with the epidermis to close the body cavity.

4. The epidermis at the anterior and posterior ends regenerates by proliferation within that layer. Mitoses are especially numerous in the ventral region adjacent to the reserve cell mass.

5. New nervous tissue arises by proliferation and inward migration of cells from the adjacent epidermis. The old nerve cells do not participate in regeneration.

6. Material for regeneration of the intestinal lining arises by proliferation within the gut epithelium.

7. Mesodermal structures regenerate from old mesodermal tissues. Replacement material is supplied by (*a*) nuclei and cytoplasm from muscle cells and connective-tissue elements after degeneration of their differentiated cytoplasm; (*b*) peritoneal cells which furnish most of the new material.

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PLATE 1

FIG. 1—Median sagittal section of regenerating worm 24 hours after severing 8 anterior segments. Eversion of the gut lining to cover the wound is shown. x 167. *bv*, blood-vessel; *cm*, circular muscles; *cœ*, cœlome; *end*, intestinal epithelium; *epi*, epidermis; *g*, ganglion cells; *lm*, longitudinal muscles; *nc*, nerve cord; *pc*, peritoneal cells; *s*, septum; *sm*, syncytial material; *x—x*, plane of cut.

FIG. 2—Median sagittal section of regenerating worm 3 days after severing 8 anterior segments. Numerous syncytial cells are present. The ventral epidermal cells have pushed anteriorly around the exposed end of the nerve cord to maintain contact with the gut epithelium. x 136. *l*, blood lacunæ; *st*, stomodæum. Other labels as in figure 1.

FIG. 3—Median sagittal section of regenerating worm 4 days after severing 8 anterior segments. x 174. *br*, cephalic ganglion; *bv*, blood-vessel; *pro*, prostomium; *ph*, pharynx. Other labels as in figures 1 and 2.

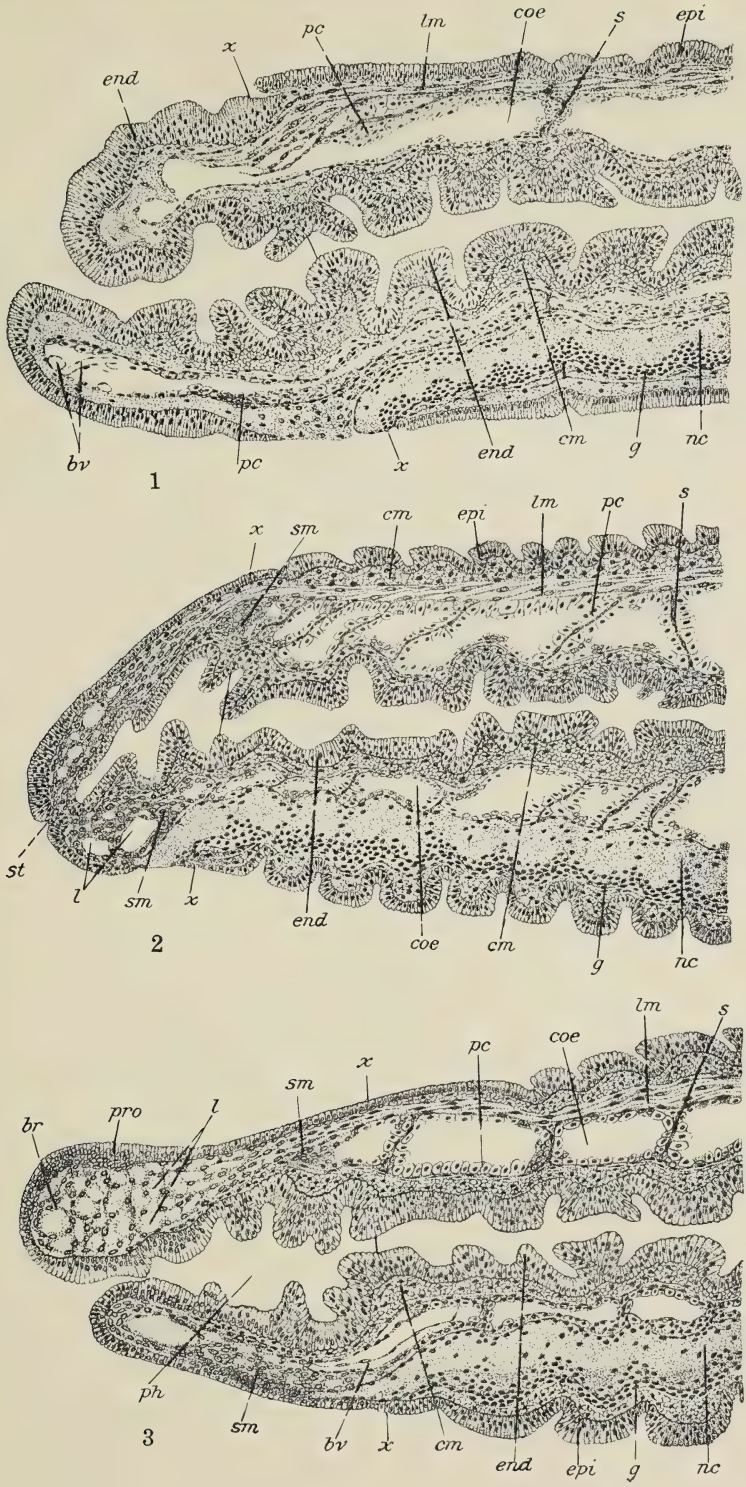
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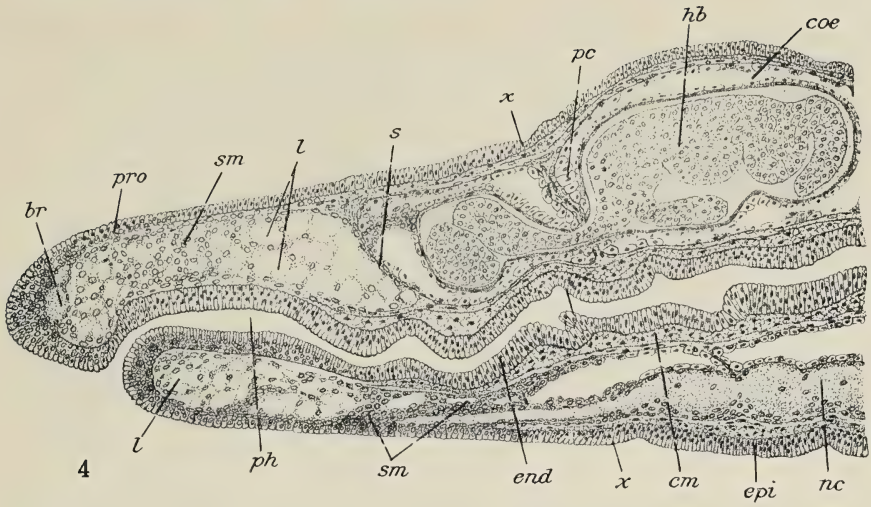
Plate 2

Fig. 4—Median sagittal section of regenerating worm 6 days after severing 8 anterior segments. x 175. *hb*, heart-body. Other labels as in figures 1 and 3.

Fig. 5—Median sagittal section of regenerating worm 15 days after severing 8 anterior segments. Regenerated region has reached maximum length. x 90. *t*, tentacles. Other labels as in previous figures.

Fig. 6—Sagittal section of regenerating region 24 hours after severing 20 to 30 posterior segments. The everted intestinal epithelium covers the wound. x 120. *a*, anus; *mc*, muscle cells. Other labels as in figure 1.

Fig. 7—Median sagittal section of regenerating region 2 days after severing 20 to 30 posterior segments. Retraction of the intestinal lining and aggregation of syncytial material are shown. x 165. Labels as in figures 1 and 6.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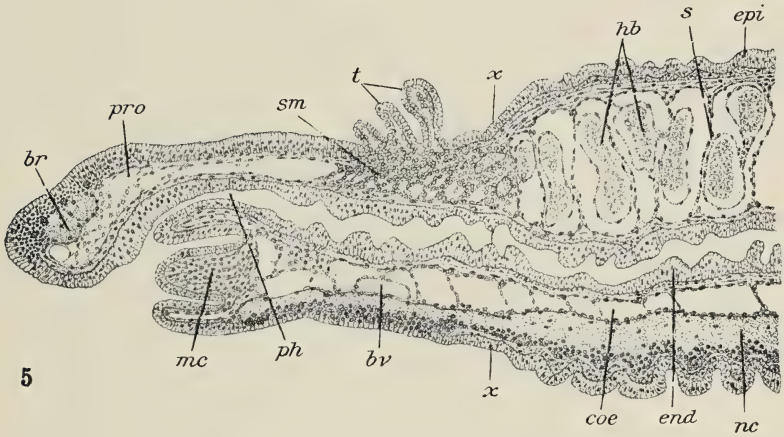
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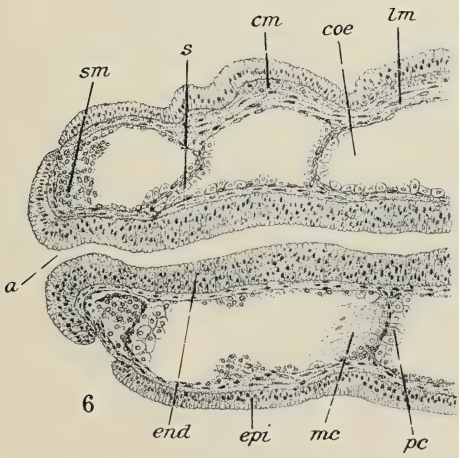
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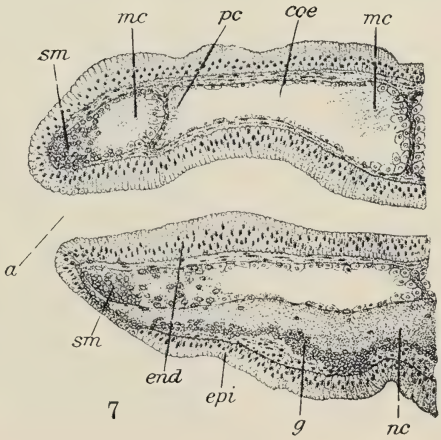
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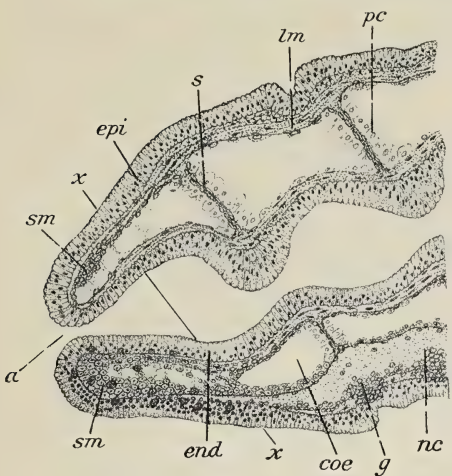
Plate 3

Fig. 8—Median sagittal section of regenerating region 3 days after severing 20 to 30 posterior segments. Origin and formation of new mesoderm from syncytial material is shown. x 168. Labels as in figures 1 and 6.

Fig. 9—Median sagittal section of regenerating region 4 days after severing 20 to 30 posterior segments. Further aggregation of syncytial material and mesoderm formation. x 162. Labels as in figures 1 and 6.

Fig. 10—Median sagittal section of regenerating region 8 days after severing 20 to 30 posterior segments. Regeneration of many segments is complete. x 195. Labels as in figures 1 and 6.

Fig. 11—Movement and aggregation of regeneration cells in terminal region 48 hours after severing posterior segments. Peritoneal cells, connective-tissue cells, and nuclei and cytoplasm from the degenerated muscle cells supply the material. The origin and appearance of regeneration material are similar at the anterior end. x 750. Labels as in previous figures.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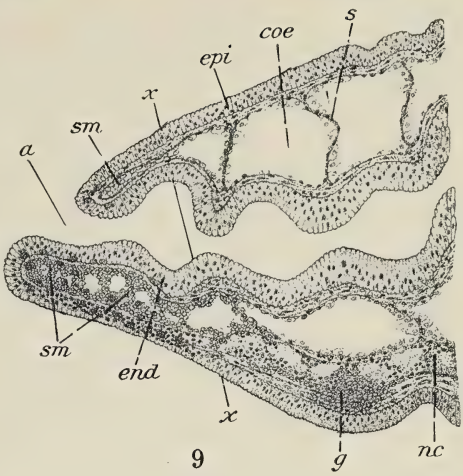
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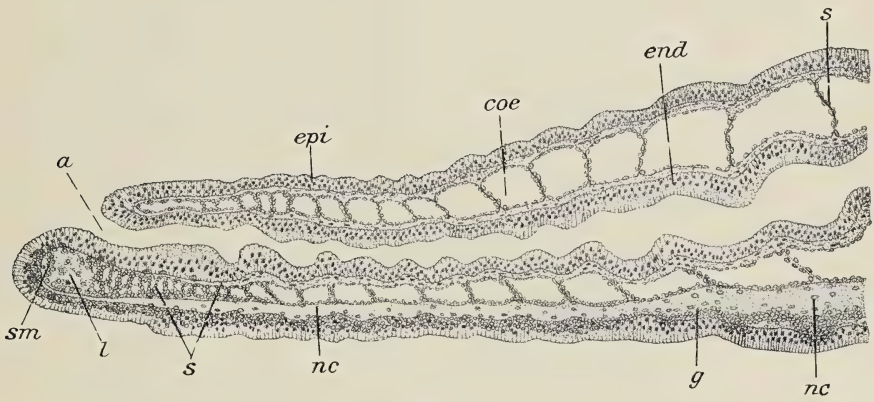
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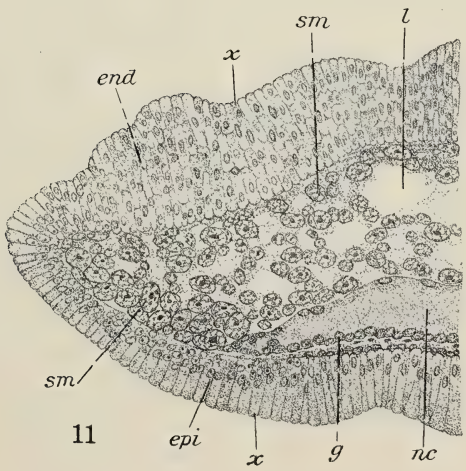
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PAPERS FROM TORTUGAS LABORATORY, VOL. XXIX

II

CYTOLOGY OF VALONIA

By Wm. L. Doyle

Zoological Laboratory of Johns Hopkins University

With one plate and seven text-figures

[Issued November 30, 1935.]

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CYTOLOGY OF VALONIA

INTRODUCTION

For a number of years algal cells with large vacuoles have been the subject of research on the permeability of the plasma membrane. Prominent among forms investigated is *Valonia*. Despite extensive literature (1, 2) the morphology of *Valonia* has had only fragmentary description (3, 4). Taylor (3) has described the habits and specific characters of the members of the group Valoniaceæ and Kopac (5) has noted the formation of aplanospores when large cœnocytes are punctured.

This paper is an account of the cytology of *Valonia ventricosa* and *Valonia macrophysa* with particular emphasis on structures of significance in physiological investigations. The cytoplasmic components to be found in cœnocytes, rhizoids and aplanospores and the relative numbers of each and their distribution are described.

The cells were collected on Bush Key Reef and from the moat at Fort Jefferson and kept in finger bowls in the laboratory. Observations were made with water and oil immersion objectives on living material at Tortugas and on fixed material at the Zoological Laboratory of The Johns Hopkins University.

STRUCTURE OF LARGE CŒNOCYTES

The cytoplasmic constituents of *Valonia ventricosa* and *V. macrophysa* are so nearly alike that both types may be considered simultaneously. The tough cell wall has been found to be cellulose (6). Immediately beneath this wall is a thin layer of protoplasm 5 to 8 μ thick (fig. 1). In surface

Fig. 1—Cross-section of cytoplasm (*c*) and cell wall (*w*) of *Valonia* cœnocyte; *n*, nucleus; *p*, plastid, cross-hatched bodies are lipoid granules. x 1100. From living material.

view (fig. 2) the arrangement of the plastids is of especial interest. A comparison of diagrams such as these reveals the fact that the plastids comprise 67 per cent of the surface area and 60 to 65 per cent of the total volume of the cytoplasm. In addition to the plastids there are nuclei and a varying number of lipoid granules suspended in the hyaline cytoplasm. The lipoid granules constitute 0.2 to 0.5 per cent of the total volume of the

cytoplasm. The nuclei are extremely numerous. There are 290 to 320 per sq. mm. of cell surface and they constitute approximately 1.2 per cent of the total volume of the cytoplasm.

FIG. 2—Surface view of cytoplasmic components of *Valonia*. Nucleus stippled, lipoid granules cross-hatched; *p*, plastids; *s*, starch. x 1650. Living material.

If we assume that the hyaline cytoplasm is the only portion of the cytoplasmic constituents involved in the transportation of water and salts, the relative proportions and distribution of the other constituents are not insignificant in the interpretation of experimental results. If this assumption is valid, only 31 per cent of the protoplasmic layer 5 to 8 μ thick is involved in the transportation of dissolved substances, and this 31 per cent is almost entirely in the nature of lamina between the plastids averaging 1.5 μ in thickness. In other words, although there is a continuous hyaline cytoplasmic surface beneath the cellulose wall, and another at the surface of the central vacuole, the main volume of the hyaline cytoplasm is a reticulum composed of relatively thin walls surrounding regions of negligible conductivity.

Puncture of the cœnocyte always results in a migration of the plastids away from the site of puncture and after healing of the puncture (by fusion of the hyaline cytoplasm), a very slow migration back to the original position. It is suggested that part of the period of low electrical resistance of the protoplasmic wall occurring after the insertion of a micro salt bridge is due merely to the scattering of the plastids leaving a relatively large area of normal hyaline cytoplasm (7).

STRUCTURE OF PLASTIDS AND ORIGIN OF LIPOID GRANULES

The plastids vary in size from 2.5 to 20 μ in diameter. They are normally blue green in color, but under adverse conditions are reddish brown, owing to the presence of phycoerythrin. Those over 10 μ in diameter contain starch grains which range in size from 8 μ downward and are usually about 3 μ in diameter. Each starch grain contains a central body of unknown nature (fig. 2 and Plate 1d). In addition to the starch grains, there are frequently found one or more lipoid granules imbedded in the plastid to one side of the starch grain (figs. 1 to 3 and Plate 1d). The granules arise in the plastids

and eventually come to be free in the cytoplasm. They are more numerous in cells kept in the dark than in those kept in the light. As found in the cytoplasm they are highly refringent, slightly yellowish spheres about 2 μ in diameter. They blacken intensely with osmic acid and are dissolved by acetic acid.

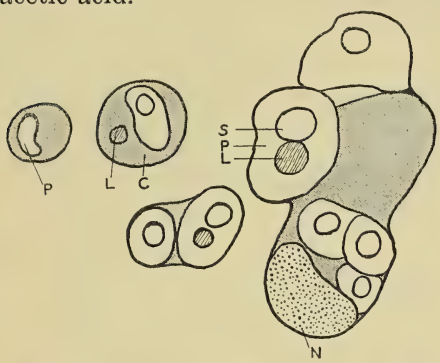

FIG. 3—Drawings of early stages in the formation of aplanospores (10 minutes after puncture of cœnocyte). *l*, lipoid granules, other symbols as figures 1 and 2. x 1500.

FIG. 4—Plastids in rhizoids in various stages of division. *Py*, pyrenoid, *pd*, dividing plastid, *m*, mitochondrium. x 750.

The plastids in the rhizoids differ from those in the cœnocyte in that they contain in addition to starch and occasional lipoid granules, a brilliant orange pyrenoid composed of one to six granules (fig. 4). Whereas only mature plastids are found in the cœnocyte, all stages in the formation of plastids from mitochondria can be seen in the rhizoids (figs. 4 and 5). In the cœnocyte plastids increase in number by division.

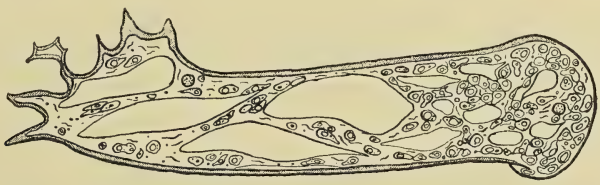

FIG. 5—Rhizoidal cell of *Valonia macrophysa* with two nuclei. x 275.

STRUCTURE OF THE NUCLEUS

As previously noted, the nuclei in the cœnocytes vary in number from one or two to hundreds of thousands, depending on the size of the cell. Mature rhizoids contain four or five nuclei.

All of the nuclei in a single cœnocyte are in approximately the same stage of chromatin condensation at the same time. The resting nucleus in a slowly growing cell is approximately 6 μ in diameter, whereas the resting nucleus in an actively growing cell is usually about 8 μ in diameter. Mitosis is intranuclear, no clearly defined spindle being formed. At telophase the nucleus constricts into two (fig. 6).

DISTRIBUTION OF RHIZOIDAL CELLS

Developing aplanospores as small as 50 μ are frequently found with one rhizoid. Spherical cells 1 to 3 mm. in diameter have three to fifteen rhizoids, and those 5 to 7 mm. have ninety to one hundred and ten. Cells of 1 cc. volume average two hundred rhizoids scattered in four to six groups of five

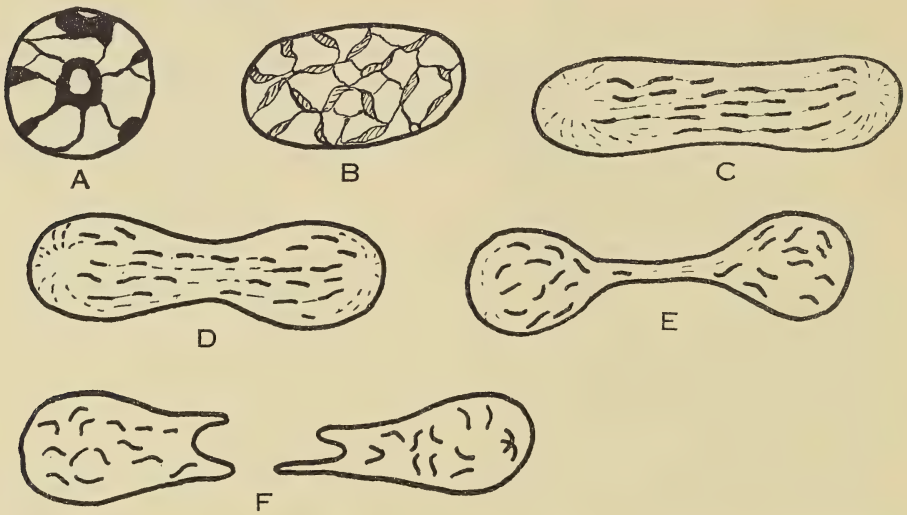

FIG. 6—Mitotic stages. *a*, resting nucleus; *b*, prophase; *c*, *d*, *e*, late prophase to anaphase; *f*, teolphase and division of nuclei. x 2650.

to sixty rhizoids per group. One cell of 10 cc. volume had over six hundred rhizoids.

Valonia macrophysa growing in mats frequently shows no rhizoids. In this situation the adjoining outer layers of the cellulose wall are common to the adjacent cells. Close examination reveals, however, that the surfaces of all cells so situated are studded with knob-like protuberances which are in reality rhizoid primordia. Upon detachment of a single cœnocyte from the mat of cells, these primordia grow rapidly into the familiar holdfasts.

Each of these primordia has one nucleus and numerous large plastids containing large starch grains and pyrenoids. There is no vacuole in the holdfast primordium in the resting condition. The development of the rhizoid from its primordium is in all essential respects comparable to the development of the rhizoid of an aplanospore (*vide infra*).

DEVELOPMENT OF APLANOSPORES

As described by Kopac (*loc. cit.*), when the cœnocyte is punctured the protoplasm leaves the cell wall and accumlates at the bottom of the sac as a mass of cytoplasm, plastids and nuclei. This mass separates into thousands of blobs each of which acquires a cell wall and begins to develop into a cœnocyte (fig. 3).

A number of such cells of *Valonia ventricosa* were placed in beakers and examined and measured from day to day. In all the cells nuclear divisions and growth occurred. Shortly after the appearance of the central vacuole, a thickening of the cytoplasm occurs at one side of the cell. The cytoplasm, plastids and nuclei of this region aggregate into a spheroidal mass and a cell wall soon cuts this mass off from the rest of the cell (fig. 7; Plate a-c). When the wall is complete, the segregated mass develops into a rhizoid.

Before taking up the changes occurring in the developing rhizoid, it is well to consider that all of the constitutents of this cell have come from a

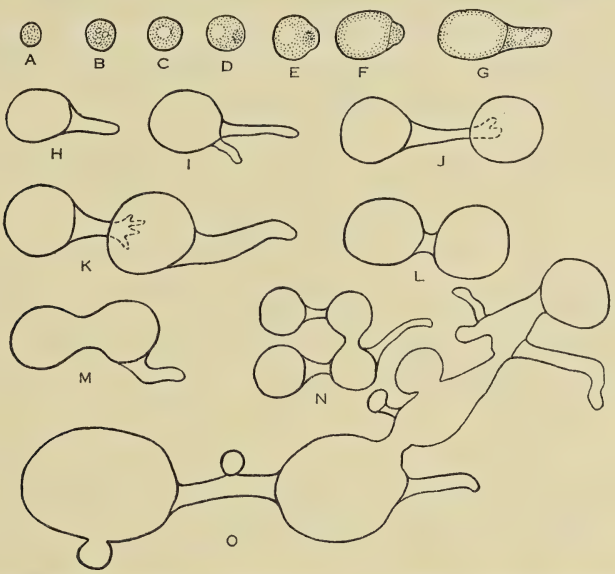

FIG. 7—Aplanospores of *Valonia ventricosa*. *a-g*, optical sections showing origin and growth of central vacuole and formation of rhizoidal cell; *h-m*, outlines of various stages in growth and fusion of aplanospores; *n* & *o*, outlines of several aplanospores fusing simultaneously. x 50.

cœnocyte which contained only mature plastids, lipoid granules, nuclei and hyaline cytoplasm.

As the rhizoid develops, the plastids undergo frequent division and also send out tenuous processes which are budded off to form mitochondria (fig. 4). There appear in the matrix of such plastids several orange granules, the pyrenoids. It would appear that in this case we have the only evidence so far presented concerning the formation of mitochondria from plastids; thus reversing the usual process of development. After the appearance of the mitochondria in the rhizoid, numerous small vacuoles also appear which fuse to form mature rhizoid structure (fig. 5).

In cells of all sizes it is observable that there is slow protoplasmic streaming in the rhizoids and none in the main cell.[1] This indication of a higher

[1] The lack of even very slow protoplasmic streaming in the main cell is evidenced by the fact that the largest starch grains are always found in the plastids on the side of the cell nearest the source of light.

rate of metabolism in the rhizoid is supported by the fact that the rhizoids frequently enter other cells and result in the fusion of aplanospores (fig. 7). After a rhizoid has entered another cell, it gradually shortens and thickens until the two main cells come into contact, whereupon the cell walls of the main cells break down at the point of contact and the contents fuse.

ORIGIN OF THE CENTRAL VACUOLE OF THE CŒNOCYTE

Minute vacuoles give rise by fusion to the large central vacuole of the cœnocyte. These were traced in sectioned preparations of aplanospores. It was possible to trace the vacuoles down to a diameter of 10 μ, at which stage they showed no evidence of a tonoplast wall or of any lipoid aggregation at the surface. We have found no evidence that these vacuoles arise in connection with any other structure in the cell.

NUCLEAR-CYTOPLASMIC RATIOS IN VACUOLATED AND NON-VACUOLATED CŒNOCYTES

Newly formed aplanospores are solid masses of protoplasm which may grow to a diameter of 71 μ before the appearance of a single vacuole. During this growth the nuclei divide regularly. It is of interest to compare the nuclear cytoplasmic ratios of vacuolated and non-vacuolated cells in order to ascertain whether or not the ratio remains fixed regardless of the presence of the vacuole.

The results of measurements on cells containing one to nine nuclei and no vacuole indicate that the number of nuclei is proportional to the volume of the spherical cœnocyte. On the other hand, in cells containing a large central vacuole surrounded by a layer of protoplasm 6 to 8 μ thick with 14 to 400,000 nuclei, the number of nuclei per cell is proportional to the surface area of the cœnocyte rather than to its volume. This area is approximately proportional to the volume of the cœnocyte minus the volume of the central vacuole or in other words to the volume of the cytoplasm. In cells with six to fourteen nuclei and small central vacuoles the ratio is the same if the volume of the vacuole is omitted from the calculation of the volume of the cytoplasm. Thus the nuclear-plasmic ratio is constant for all sizes of cells if the volume of the central vacuole is deducted from the total volume of the cell. In the cells the nuclei are very regularly and evenly distributed throughout the cytoplasm.

EXPERIMENTS WITH VITAL DYES

In identifying the various structures in the cœnocytes and rhizoids of *Valonia,* neutral red and janus green were employed in various concentrations.

In any cell the cellular components stained by vital dyes may be divided into two groups: those present in the cell before the stain was applied and the artifacts arising from the reaction of the cell to the staining solution.

In the main cell the lipoid granules and the vacuolar fluid were stained with neutral red. No structures stained by janus green were observed. In the rhizoids, on the other hand, the lipoid granules were stained with neutral red and the filamentous mitochondria with janus green. It was impossible to ascertain whether or not the limiting boundary of the large vacuole of the main cell was stained with janus green. No other structures normally present in the cells were stained by these dyes.

When cells were treated with fairly high concentrations of the dyes, however, it was noted that vacuoles were formed in the cytoplasm of the rhizoids. These vacuoles were homogeneously stained red when neutral red was employed and were found to have their walls stained green when janus green was used. If both dyes were applied simultaneously the vacuoles resulting were dark colored and consisted of a red fluid enclosed by a wall stained green. These staining reactions of artifacts produced in this manner are strikingly reminiscent of the staining of the normal insect spermatid acroblast.

SUMMARY

The morphology of the various structures in the cytoplasm of *Valonia macrophysa* and *V. ventricosa* is described. The plastids produce starch and lipoid granules and are sufficiently numerous as to constitute two-thirds of the volume of the cytoplasm. There are approximately three hundred nuclei per square millimeter of cell surface in the cœnocytes. Mitosis is intranuclear.

In the development of the rhizoidal hapteron cells of the aplanospores, the mitochondria arise from plastids of the cœnocyte in which the aplanospores were formed.

The large central vacuoles of the cœnocyte arises by fusion of small vacuoles formed in the cytoplasm.

Double vital staining of artifact vacuoles is noted.

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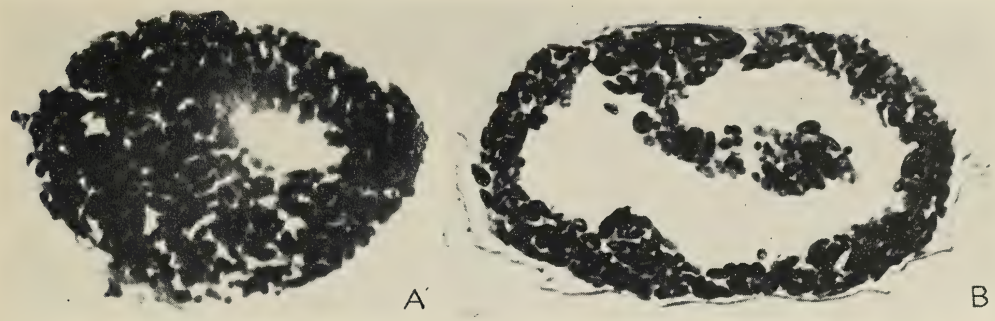
Description of Plate 1

a and *b*, Sections through developing aplanospores showing enlargement of central vacuole. Champy Fixation—Hæmatoxylin 4μ. *c*, As above showing aggregation of cytoplasm at one side preparatory to formation of rhizoid. *d*, Section through cytoplasm showing plastids and lipoid granules. Plastids contain starch and developing lipoid granules can be seen surrounding starch grains. Starch shows a central granule. Champy fixation. Unstained 4μ. a, b, c, x 422; d, x 1930.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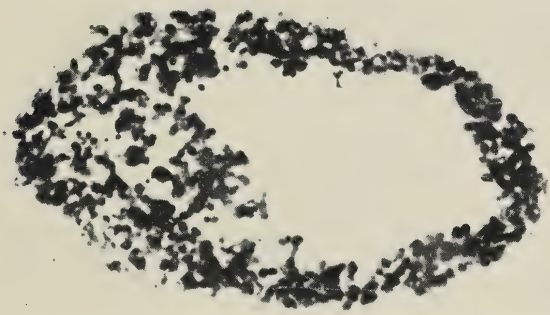
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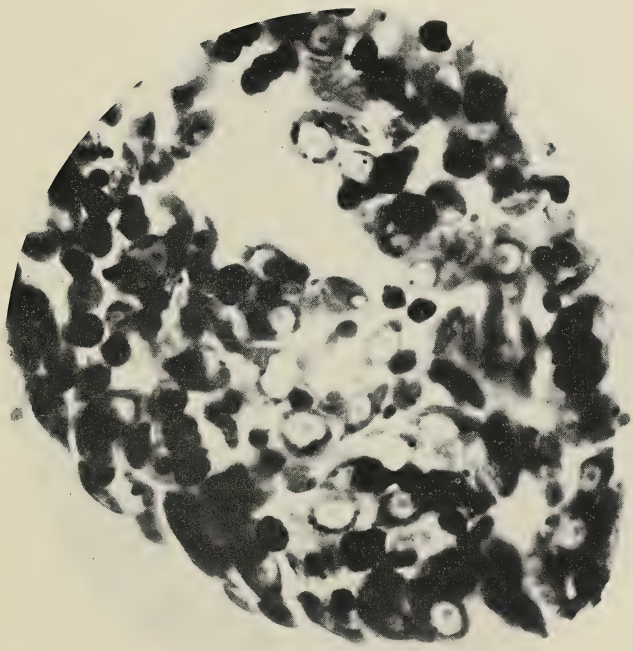
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PAPERS FROM TORTUGAS LABORATORY, VOL. XXIX

III

EFFECT OF LIGHT ON ORIENTATION AND STABILITY OF YOUNG PLECTOGNATH FISH

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and
J. E. Harris
University of Cambridge, England

With three text-figures

[Issued November 30, 1935.]

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EFFECT OF LIGHT ON ORIENTATION AND STABILITY OF YOUNG PLECTOGNATH FISH

INTRODUCTION

Under certain circumstances, young forms of some plectognath species, and to a lesser extent representatives of other groups, will respond to a strong beam of light by violent gyrations. This was first observed by Breder (1929) at the Tortugas Laboratory of the Carnegie Institution of Washington, and the results were more recently confirmed and extended by Harris (1934). The mechanism by which these movements are effected, their relationship to the intensity and duration of the stimulus, and the disappearance of the phenomenon with advancing age of the animal give rise to a number of interesting problems concerning the action of the receptor-effector system in these fish. The present communication is an attempt to explain this feature of fish behavior, so far as is possible at this time. Doubtless, a more detailed study of the problem would yield information of much interest and value to the student of animal behavior. The general features have already been sketched in an abstract (Breder and Harris, 1934), but the detailed observations, which in some ways may be considered the most suggestive, have been reserved for this contribution.

The authors wish to express their appreciation to Dr. W. H. Longley for his friendly interest and able assistance in obtaining material for the studies.

EXPERIMENTAL STUDIES

DESCRIPTION OF PHENOMENA

Unless otherwise mentioned, the description refers to the fish *Monacanthus (Stephanolepis) hispidus* (Linnæus). The peculiar gyrations are best initiated by the play of a common electric focusing flash-light on a tank placed in a darkened room. The fish may react immediately, but usually there is a delay before the rotation commences, and the primary response would seem to be an attempt to face the dorsum toward the light.

The most usual motion is one of rotation about a transverse axis, best described as "somersaulting" since the axis is not coincident with the center of area of the fish as seen in side view, but may be above or below this point. In specimens which somersault slowly, it can be seen that the mechanical origin of this movement lies in the transmission of undulations along the dorsal and anal median fins, which normally serve as the organs of locomotion. The waves propagated in these fins are seen to move in opposite directions. The resulting translational force is negligibly small,

but a large rotational couple is produced (fig. 1b). The rotation may be in either direction, dependent on which way the anal and dorsal waves travel.

It is also quite common for the fish to rotate about a longitudinal axis. Such a motion is always accompanied by a considerable forward movement, and has been found to be due to the bending of the anal and dorsal fins to

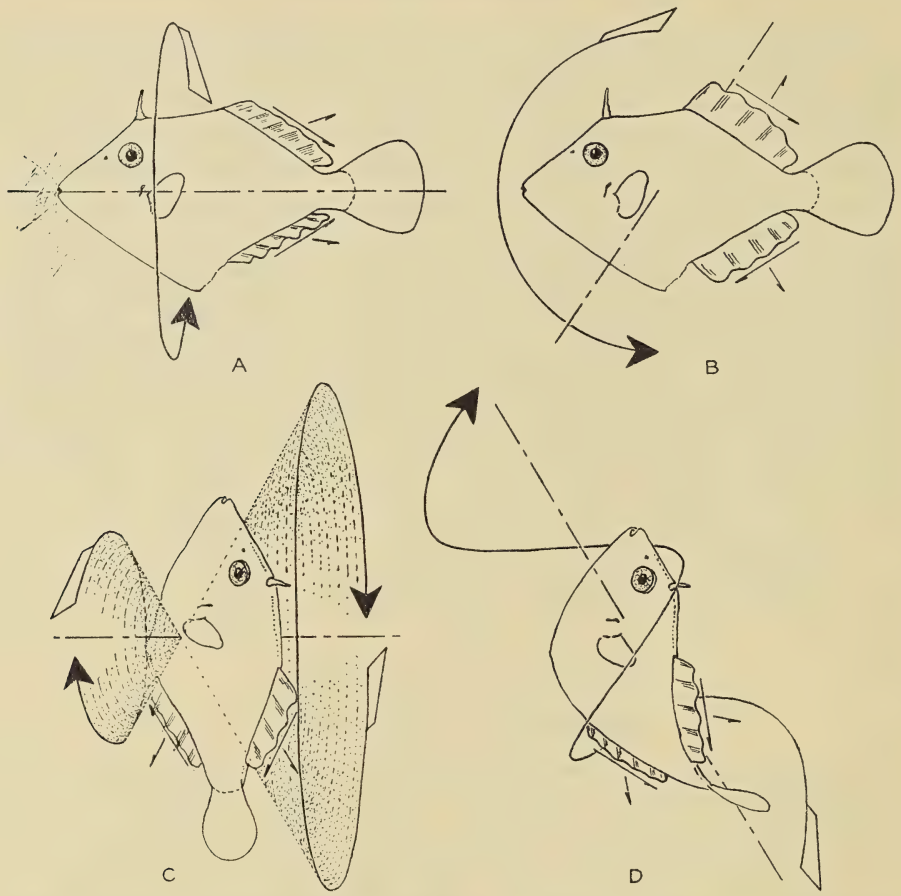

FIG. 1—Motion of *Monacanthus* under influence of strong light beam. In each case feathered arrows indicate movement of fish as whole. Arrows parallel to dorsal and anal fins indicate directions of travel of propulsive waves. Arrows parallel to the fin rays indicate direction of radial component. A, rolling about longitudinal axis; B, typical somersaulting; C, unstable condition of rotation leading to true somersaulting, as in B; D, helical path produced by fin movement as in A, but with flexed tail.

opposite sides of the body, while transmitting the undulations anteroposteriorly in both fins. There is thus a radial component of the fin action as well as a forward one, and the fish revolves while being driven forward (fig. 1a). On reaching the glass wall of the aquarium, the fish spins on its pointed nose in a toplike fashion. Should the axis of the fish become inclined toward the wall a precessional movement ensues, exactly similar to that of a gyroscope supported at a point other than its center of gravity.

The conditions, of course, are strictly comparable, the pull of gravity on such a gyroscope corresponding to the translational propelling force being exerted by the fin structures.

As would be expected from these observations, amputation of one or both of the pectoral fins does not prevent such gyrations (indeed, it may even initiate rapid gyrations in *Lactophrys* which have been repeatedly stimu-

TABLE 1—*Gyration of non-blinded fish. Combined observations (1929 and 1934)*

Species	Nos. neg.	Nos. pos.	Sizes[1] negative	Sizes positive	Remarks[2]
Monacanthus hispidus	4	50±	20(1) 7(2) 50(1)	15-50	7 mm. too small? 50 mm. too large
Lactophrys triqueter	5±	50±	70	15±	Especially violent
Lactophrys bicaudalis (?)	0	1	...	15	Especially violent
Lactophrys trigonus	0	7	...	60±	
Lactophrys tricornis	1	1	100±	100±	Reaction very delayed
Hæmulon sciurus	50+	4	30	30	Rotated on long axis
Pomacentrus leucostictus	8	2	20(4) 25(4)	25(1) 20(1)	1 fish (25 mm.) back to light only 1 fish (20 mm.) reacted violently
Hemiramphus brasiliensis	30+	1	8-35	25±	Spun on its beak, against glass
Sparisoma radians	0	15±	...	60-100	Very irregular spiral gyrations

Other species always found to be negative:

Cypselurus furcatus	*Calamus arctifrons*	*Syngnathus mackayi*
Scarus sp.	*Synodus fœtens*	*Apogonichthys* sp.
Callionymus sp.	*Calamus (calamus* or *proridens)*	*Alutera* sp.

[1] Numbers in parenthesis indicate number of individuals. Other numbers indicate lengths in mm. without caudal fin.

[2] Unless otherwise indicated a positive reaction implies somersaulting.

lated by exposure to light), but the removal of the dorsal and anal fins eliminates the phenomenon entirely. Removal of a single one of the unpaired fins permits slow gyrations, accompanied by a considerable translational motion. The relationship of these phenomena to the normal response to light is considered in the discussion.

In certain exceptional cases, a third type of motion has been observed; the body of the fish traveling along a helical path, and rotating on its longitudinal axis while so doing. The movements are so rapidly performed

that detailed observation of the mechanism of this rotation is almost impossible, but it may be pointed out that if the tail be flexed while the animal is performing a series of rotations about a longitudinal axis, the resultant path would be precisely a spiral of this type (fig. 1d). The directions of motion of the median fins have not been fully followed in this case, but the flexure of the tail is very obvious, and the above explanation is therefore probably the correct one. Figure 1c represents an unstable condition of motion and will be treated in the discussion. All three types of movement have been observed in several species of *Lactophrys*, with appropriate modifications in speed and character, due to the almost spherical body form of the small specimens studied, and the difference in size and type of the median fins from those of *Monacanthus*.

The speed with which these gyrations are sometimes performed is almost incredible. Small specimens of *Monacanthus* have been known to somersault as rapidly as 180 rotations per minute, though their rate of rotation about a longitudinal axis is considerably slower. This difference would be expected on consideration of the laterally flattened body form of the animal, which would greatly facilitate any motion in its own plane, while increasing considerably the resistance to any form of transverse motion, necessarily implied in rotation about the longitudinal axis of the fish.

The mechanics of the gyrations of *Hæmulon*, *Pomacentrus* and *Hemirhamphus* are more difficult to elucidate, as their behavior was too erratic and too seldom observed for the details of the movements to be clearly seen. In the case of the parrotfish *Sparisoma radians* (Cuvier and Valenciennes), the phenomenon is sufficiently common to enable some of its characteristics to be noted. The primary reaction to the stimulus in these forms, as in the plectognaths, is to orient themselves so that the light is incident dorsally upon them. In the aquarium, *Sparisoma* usually rests on the bottom of the tank, and any reorientation therefore commences with a normal forward swimming. The pectoral fins are inclined in such a way as to rotate the animal upward and to roll it into the correct position with respect to the light source. The natural consequence of the sudden start is invariably an overcorrection, and the fish proceeds to execute a large number of rotations about its longitudinal axis, coupled with a helical translational path such as that described in the third type of rotation in *Monacanthus*.

DEVELOPMENT OF RESPONSE

The somersaulting response is rarely, if ever, obtained from fish which are taken straight from daylight into a darkened room and subjected to a light beam, however intense it may be. There is apparently a period during which the sensitivity to light stimuli is gradually built up until the characteristic response is obtained at its full strength. In fact the process is sufficiently well defined to be divisible into three more or less distinct phases.

1. For some hours after the fish is first placed in the dark room, the reaction to light stimuli is almost inappreciable. However, during this stage, which usually lasts from four to six hours, there is an increasing tendency for the fish to orient itself with respect to the source of light, turning itself in a horizontal plane until the eyes are facing the light. There is also a distinct rolling of the animal about a horizontal axis, so that the dorsal surface tends to be directed toward the light beam.

2. This is the stage when the reaction of gyration is most clearly defined. In *Monacanthus hispidus* the sensitization rarely passes this second stage, which is characterized by a delayed but very definite, positive response to the presence of an illuminant, ceasing very shortly after the light source has been extinguished. In the earlier part of stage 2, the animal usually commences to somersault about 30 to 60 seconds after the light is switched on, and it may perform only a few turns before coming to rest again. Very shortly, however, with the tank still illuminated, a new set of somersaults may be initiated which again may cease after about a dozen rotations have been performed. Later, in stage 2, these intermittent responses give place to a single uninterrupted gyration continuing until the light is extinguished, though there is still a delay of about thirty seconds before the light produces the initial response.

3. In the case of *Lactophrys triqueter* (Linnæus) and *L. trigonus* (Linnæus) the animal seems to become progressively more and more sensitive to the light stimulus. It soon commences to react immediately on application of the stimulus, and frequently fails to stop on extinguishing the light. Deep red light, to which the animal is not sensitive in stages 1 and 2, may produce just as violent a reaction and finally the fish may become so completely disorganized as to go on spinning for hours without a stop. Any stimulus, such as vibration, the fin amputation previously noted, the tapping of the tank, as well as a light beam, will initiate gyration in such sensitized fish. The equilibrating system of the fish seems to become completely disorganized. Continued gyration, for periods of time exceeding a few minutes, has not been noticed in *Monacanthus*, and at no time was it found possible to stimulate them to gyrate by means other than a beam of light.

A summary of the experiments of both authors, made on two occasions, would indicate the following results. Somersaulting was never seen in blinded fish, unless they had been previously "dark conditioned." A single specimen of *Monacanthus hispidus*, which was unilaterally blind when caught, swam normally. Specimens of *Monacanthus* taken straight from a brightly illuminated tank, if blinded in both eyes by covering them with a vaseline lampblack mixture, usually showed a complete loss of all sense of orientation and swam upside down or on their sides, indifferently. *Lactophrys* subjected to the same treatment did not seem to lose its sense of equilibrium but swam right side up with little difficulty. This is curious when one notes the extreme loss of equilibrium displayed by long dark-sensitized

(stage 3) *Lactophrys*. There was no tendency to gyrate. Unilateral blinding of such a fish produced turning movements toward the side of the unaffected eye, thus showing the same phototropic orientation as when subjected to a light source in the darkroom (fig. 2). When the single lampblack patch fell off, normal swimming was resumed.

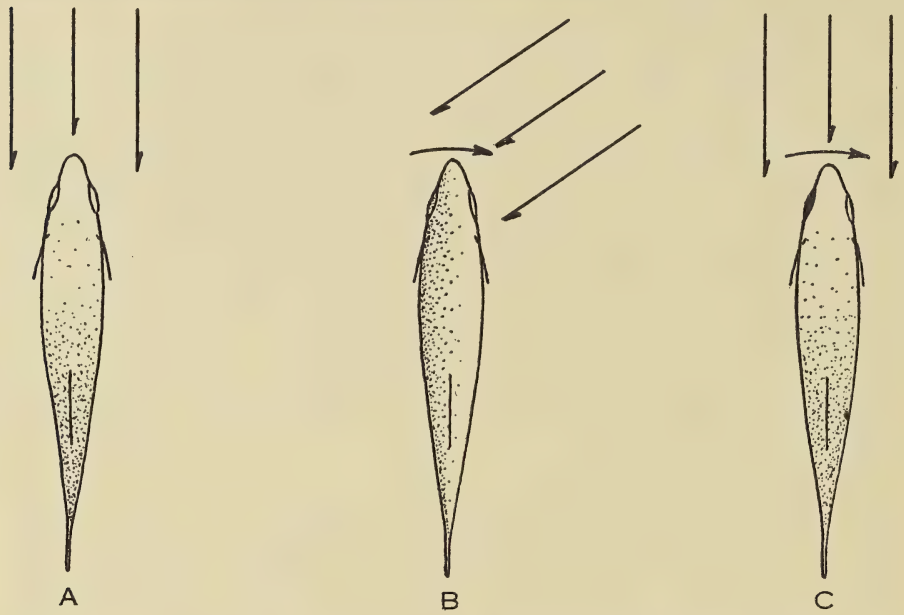

FIG. 2—Turning under influence of positive phototropism. A, light ahead of fish; B, light to one side; C, one eye blinded. Note that effect in C is similar to that in B.

On the other hand in a fish which had been confined to a darkened tank for some hours previously, unilateral blinding produced typical gyratory movements. Orientation in the horizontal and vertical planes was almost invariably followed by such gyrations, which ceased immediately the patch fell off the blinded side.

In the case of *Lactophrys* which had been confined in the darkened tank for a considerable period, blinding in one or both eyes was always accompanied by gyrations. That this was merely due to the shock effect was shown by the fact that even if the patches failed completely to stick on, the fish still gyrated frantically and for a very long time. Such a reaction has already been described as typical of stage 3 of the sensitization.

DISCUSSION

It seems obvious that the gyrations described in the preceding sections can have little or no survival value for the species. In fact it seems to be definitely established that violent or continuous changes in the orientation of a fish is decidedly harmful, if not indeed fatal to the fish. The incipient

instability of the fish body form (the apparent equilibrium being a finely balanced steady state), extremely sensitive as it is to every little water movement around it, would seem to be a cumulative factor in its destruction, as indicated by the work of Parr (1927a). This author fixed an overturned sturgeon (weak from too much handling) in such a way as to force it into a normal position. This fish eventually recovered, but others not so treated almost invariably died, a fact which he considered was due to pressure changes produced by the reorientation of the circulatory system. Whatever may be the correct interpretation of this observation, it would seem that an organism in which pathological changes tend to produce abnormal orientation, which in its turn induces further pathological change, is in a very insecure position with regard to its possibilities of survival even under favorable circumstances.

It would therefore appear more reasonable to assume that the disturbances are to be regarded as the results of normal automatic responses acting under abnormal conditions of presentation of the exciting stimuli, and that the actual gyratory motion was the product of mechanical instability consequent upon the unusual orientation induced by this response.

It is well known that normal orientation of land vertebrates can be established by three independent systems of receptor organs, *viz,* the visual, otic and proprioceptor systems, and that any or all of these three may be concerned in the maintenance of an erect position. In the fish, it seems probable that the proprioceptor system can at best only serve as a very inefficient indicator of orientation, since the body form is suspended in a medium of almost identically the same specific gravity, and the tension of the muscular system will in consequence be inappreciably different in the normal and abnormal positions. This of course is not so in the case of the land vertebrate: a man standing on his head, by reason of the altered weight distribution will have different muscle tensions from those of a man in the normal upright position, and the fact that he is standing on his head will be appreciable even apart from the sensory stimuli derived from his eyes or otic capsules. It seems also likely that the internal afferent system (*e.g.* the sympathetic nervous system) would be little acted upon by orientation changes in the fish, since it is continuously being subjected to environmental shifts; for example, wave action, changing vertical currents and other water movements. Hence it is probable that the orientation of the fish in the water will be determined by sensory stimuli derived almost solely from the eyes and "auditory" organs. That the eyes are not always necessary for the maintenance of equilibrium is sufficiently evident from the fact that various experimentalists have blinded fishes with vaseline and lampblack and obtained no evidence of disturbance of equilibrium; *e.g.* Parker (1909) *Mustelus*, Parr (1927b) working with mackerel, Spooner (1931) with a bass, and Breder and Nigrelli (1935) working with sunfishes. Yet it is obvious that in these young plectognath forms the direction of the source

of illumination is a factor of very great importance for the orientation of the animal. In *Lactophrys* the otic capsules can still preserve the equilibrium of the fish when the animal has been blinded completely, but partial blinding results in a rolling over which tends to equalize the intensities of light falling on the two sides; *i.e.* the fish rotates so that the dark side tends to face the light. But this effect is possibly counteracted by the vertical force of gravity acting on the statocyst, and the final result is a compromise between the two influences, as indicated in figure 3. As would be expected, the influence of light in disturbing the normal orientation is a direct function of the intensity of illumination; it was found that a weak light had very

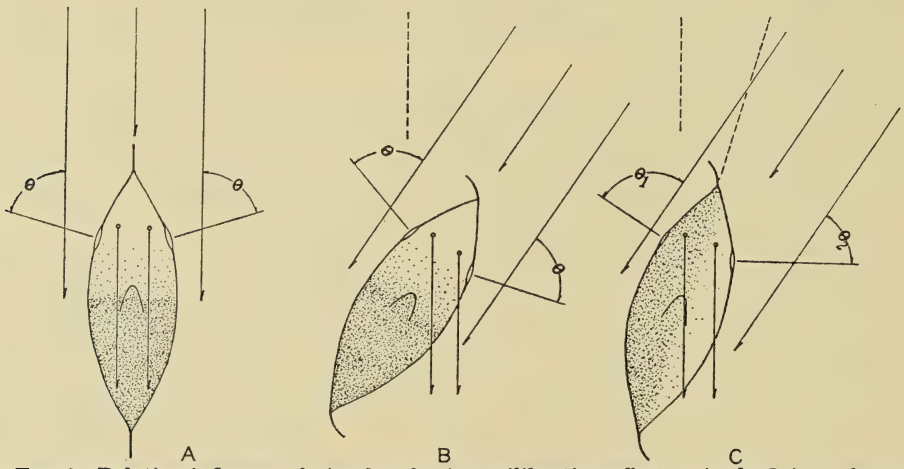

FIG. 3—Relative influence of visual and otic equilibrating effects. Angle Θ in each case determines relative intensity of illumination of the two eyes. Gravitational vector is indicated by vertical lines drawn from otic capsules. A, light vertical and parallel to gravitational pull; B, light at an angle assuming no gravitational influence; C, actual effect with resulting position, intermediate between A and B in which Θ_1 and Θ_2 are unequal.

little effect either in rolling the fish or in causing it to gyrate, a strong light made both reactions very pronounced, and also that the time taken for the fish to respond to the stimulus was longer for the weaker stimulus than for the stronger. The best measure of this was the time required to initiate somersaulting, which in some cases was as high as 180 seconds when the light intensity was only 50 candle meters, but 15 to 20 seconds with an intensity of illumination around 1000 candle meters in magnitude.[1]

Specimens of *Monacanthus* over 60 mm. in standard length never showed the somersaulting reaction, and the change in orientation produced by a

[1] These high intensities of illumination do not appear to have any harmful effect on the fish. Certainly if any such effect is existent it has never been detected. The numerous species at the New York Aquarium show little response to lateral illumination. Some will head into it, but by far the greatest majority pay little attention other than the customary uneasiness that always attends specimens which are being worked with. Some have even been noted to come to rest across a strong arc light beam, with one eye directed to the light source—an illumination which no human eye could endure without injury. Subsequent behavior demonstrated that their vision was as acute as before.

light beam was inappreciable. *Lactophrys* was found to react at a much larger size than this, but much more slowly and indefinitely than the smaller individuals, and in all the species which reacted positively to the light source the smallest ones reacted most violently. The most logical explanation of this fact would seem to be that in the orientation of the young of these fish the influence of light preponderates, and the stimuli derived from the statocysts of the otic capsules are proportionately weaker. Later in life, the center of the orientation mechanism shifts from the visual to the otic region. It may be pointed out that in the development of the embryo fish, the otic capsule is differentiated much later than the optic region, and the later elaboration of this organ suggests that it would be likely to come into use at a later stage in the life of the animal. It is, however, rather unexpected to find that the differentiation in ontogeny of these organs persists as a physiological differential for so long after the larval stage has been completely outgrown.

Disturbances in equilibrium caused by unilateral blindness, induced experimentally or pathologically, have frequently been observed. Parker (1909) failed to find any such abnormality in experimentally blinded dogfish, but many species of teleosts appear to be affected. Lyon (1909)[1] found that unilateral blindness was followed by a pronounced inclination toward the uninjured side, and this result was confirmed by Pearcy and Koppanyi (1925) and Main (1928), working with goldfish, *Carassius auratus*. Main, in the same communication, gives confirmatory results obtained with *Amphigonopterus aurora*. The workers previously referred to as using vaseline lampblack did not employ unilateral blinding.

Polimanti (1912) and Rizzo (1930) have also studied this question, but their results differ from the foregoing in that they state that the tilting is always toward the blind side. Polimanti made his observations on *Trigla* sp., *Apogon nex* and *Labrax lupus*, all suffering from unilateral exophthalmia, while Rizzo studied the effect of experimental lesions on *Julis vulgaris* and *J. giofredi*. They suggest that the reaction observed is one which favors rapid flight on the approach of enemies by increasing the effective visual field of the uninjured eye, pointing out that such fish usually rest at the bottom of the tank. While the work of the present authors confirms the results of Lyon and Main, and appears contradictory to those of Polimanti and Rizzo, it must be admitted that the reversal of phototropic reactions during ontogeny is by no means uncommon. *Trigla* and the labrids are typically bottom living forms, while *Monacanthus* and *Lactophrys* spend most of their time in active cruising about the weeds among which they live when young, or later in relatively open water. Habitat may therefore be an important consideration in interpreting these differ-

[1] Lyon worked with *Fundulus* in his earlier study, Amer. Jour. Physiol. vol. XII, 149-161, 1904 (1905). Main (1928) states that Lyon used the same fish in his work of 1909. Reference to Lyon (1909), however, does not confirm this, as he found *Fundulus* an unsuitable subject and used other species (scup, perch and sunfish).

ences. In any event, the influence of light on orientation seems to have been firmly established. Whichever the side to which the blinded fish is inclined, it is clear that dorsal illumination favors, and certainly is the controlling factor in the normal vertical position, of some species at least.

Hollister (1934), in studying the effects of a salt-water environment on *Lebistes reticulatus*, found that succeeding generations in that medium tended to swim in spirals and that "This occurs regularly when the fish are startled and lasts for some seconds after the first stimulus. The sudden flash of a light will start all the fish swimming in spirals, some more violently than others." This suggests a hyper-sensitization similar to that previously described, but induced by chemical means.[1]

It is strange that fish in the New York Aquarium have never been seen to gyrate in this manner. The sudden flashing on of a strong light may cause an uneasiness and in some cases excited movement, but never, in a period of some thirteen years, has a disturbance of equilibrium attributable to light stimuli been noted.

The phenomena so far considered are those of the change in orientation under the influence of a directional light stimulus. This does not explain the curious somersaulting reactions obtained in the later stages of reaction to light. Yet it seems probable that there is a close connection between the two. Somersaulting is always preceded by attempted orientation, and only in the later stages (hyper-sensitized *Lactophrys*) can it be produced by stimuli other than light. The other factor which must be considered is that in the plectognaths, where the somersaulting is most marked, the locomotor system is of a very highly specialized type, quite unlike that of most other teleosts. Control of the rolling movements of the fish has been shown by Harris (1934) to lie almost entirely in the dorsal and anal fins; their amputation results in almost complete loss of rotational stability about a longitudinal axis. But these fins are the ones which are employed in locomotion—their power of rolling the animal depending on their activity, and not alone on their position, as in a rudder. In other words a rolling movement can only be produced if a translational force is produced simultaneously. There are two possibilities available for the fish; the translational motion can be in the same direction in both fins, or in opposite directions. In the first case, the fish will progress forward (or, could conceivably move backward if the waves were postero-anterior in direction, although this complete reversal has never been actually observed), and in the second case somersaulting about a transverse axis will ensue. Somersaulting under such circumstances (with the body of the fish not in a vertical plane) is not a condition of stable rotational equilibrium; it can be shown that the plane of rotation will gradually change until it coincides with the vertical plane

[1] This behavior is probably only superficially similar to the spasms described by Breder (1934) on the introduction, under certain conditions, of marine fish to fresh water. The latter is a tetanus-like behavior and, so far as known, is not induced by light variations.

of revolution—it will, therefore, be ultimately independent of the direction of illumination, which is found to be the case. Figure 1c represents such an unstable condition. The fish is traveling over the surface of a cone whose vertex is the center of rotation of the animal. In such a condition the vertical angle of the cone increases until the fish is somersaulting in a plane, as in figure 1b. The light stimulus merely starts the rotations; the subsequent gyrations are determined solely by the dynamics of the system. If to these somersaulting movements is added the turning in a horizontal plane (yawing) produced by a deflection of the tail, all of the complicated spiral paths which more rarely occur are easily explained.

The slower response exhibited by older fish to violent changes in light intensity enables these larger forms to escape the fault of over correction implied in the above description, and somersaulting is rarely seen in such adult specimens.

SUMMARY

1. Small specimens of *Monacanthus* and *Lactophrys*, if exposed to a beam of light, frequently exhibit somersaulting or rotational movements of great rapidity.

2. Somersaulting is produced by passing the locomotor waves in opposite directions along the dorsal and anal fins.

3. The rotational movement is accompanied by the deflection of the dorsal and anal fins to the opposite sides of the body, the direction of motion of the undulations being usually antero-posterior in both fins.

4. Various combinations of fin and tail movements may occasionally give other twisting gyratory movements.

5. The primary response to light is always an attempt at reorientation of the animal so that the light is incident upon the dorsal surface.

6. In fish kept in complete darkness and "sensitized" by repeated stimuli, gyrations may continue after the light has been removed, and even mechanical stimuli may initiate similar paroxysms, the equilibrating system apparently being more or less permanently deranged.

7. Specimens of *Monacanthus* over 50 mm. in length do not usually display this behavior, and species other than plectognaths show it very feebly or not at all.

8. The integration of gravitational stimuli into the behavior pattern is apparently not perfectly attained until a comparatively late stage in development, and light is the primary orienting factor.

9. The gyrations are apparently due to instability, consequent upon overcorrection.

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PAPERS FROM TORTUGAS LABORATORY, VOL. XXIX

IV

CALCAREOUS SHALLOW-WATER MARINE DEPOSITS OF FLORIDA AND THE BAHAMAS

By Eldon Marion Thorp

With Appendices

1. Diatoms in Bottom Deposits from the Bahamas and the Florida Keys. By Albert Mann.

2. Current Measurements along the Florida Coral Reef Tract. By T. W. Vaughan.
   Notes on Current Observations, Florida Keys, June, October, November 1914. By Frank J. Haight.

[Issued December 9, 1935]

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CALCAREOUS SHALLOW-WATER MARINE DEPOSITS OF FLORIDA AND THE BAHAMAS

INTRODUCTION

REGIONS INVESTIGATED, AND REVIEW OF PREVIOUS STUDIES

In that region which bounds the Straits of Florida there exist many complex problems of marine sedimentation which have interested and to some degree perplexed students of geology and oceanography for over 80 years. Since 1851 when Louis Agassiz observed the fine-grained muds and oozes on the bottom underlying the shoal waters of Florida and the Bahamas there have been many distinguished names on the list of workers. Pourtalès, Alexander Agassiz, Dall, Murray, Sanford, Vaughan are but a few of them. Dall (1892) is credited by Vaughan as being the first to suggest that "much of the limy deposits of the area behind the reefs and defended by them is probably a result of the deposition of lime originally in solution and precipitated by chemical action rather than mechanically transported sediment." Sanford (1910) likewise decided that these oozes were in part chemically precipitated. Beginning in 1908, Vaughan initiated the most intensive and careful study of the shoal-water bottom deposits of Southern Florida and the Bahamas. One of his associates, Drew (1914), found that ammonia was a metabolic product from a certain group of bacteria.

Drew (1914) and others thought that bacteria were fundamentally responsible for upsetting chemical equilibria and thereby causing precipitation of calcium carbonate, but this view was attacked by Lipman (1922, 1924, 1929).

During the progress of the research it became obvious that no solution of the problem of the probable rôle of bacteria in causing the precipitation of calcium carbonate in sea-water was possible until the physico-chemical factors were understood and quantitatively evaluated. The results from such a study have applications to other problems in the ocean besides those immediately connected with marine bottom deposits. Consequently attention for the time was largely turned away from bacteria and directed toward the solution of the imperfectly understood physico-chemical relationships.

Dole (1914) analyzed sea-water which flows into and out of Tortugas Lagoon and found that although both carbonate and bicarbonate ions were present, there was no free carbon dioxide; thus indicating that calcium carbonate would not be dissolved. He and Chambers (Dole and Chambers, 1918) found the average salinity of sea-water near Fowey rocks to be about 35.90 per mille for 388 determinations made between September 12, 1914,

and October 17, 1915. This average was believed to be approximately correct for most off-shore waters of the Florida reef tract.

A sample of sea-water collected by Vaughan (see 1918, p. 273) at station 177 near the west entrance of South Bight had a salinity of 38.86 per mille, while two specimens from Cocoanut Point had salinities of 36.4 and 36.6 per mille, respectively. Drew (Vaughan 1918, p. 273) reported surface salinities of 36.24 per mille six miles east and of 36.58 per mille 13 miles east of Golding Cay, Andros Island. All available information shows the salinity values to be higher on the west side than on the east of Andros Island.

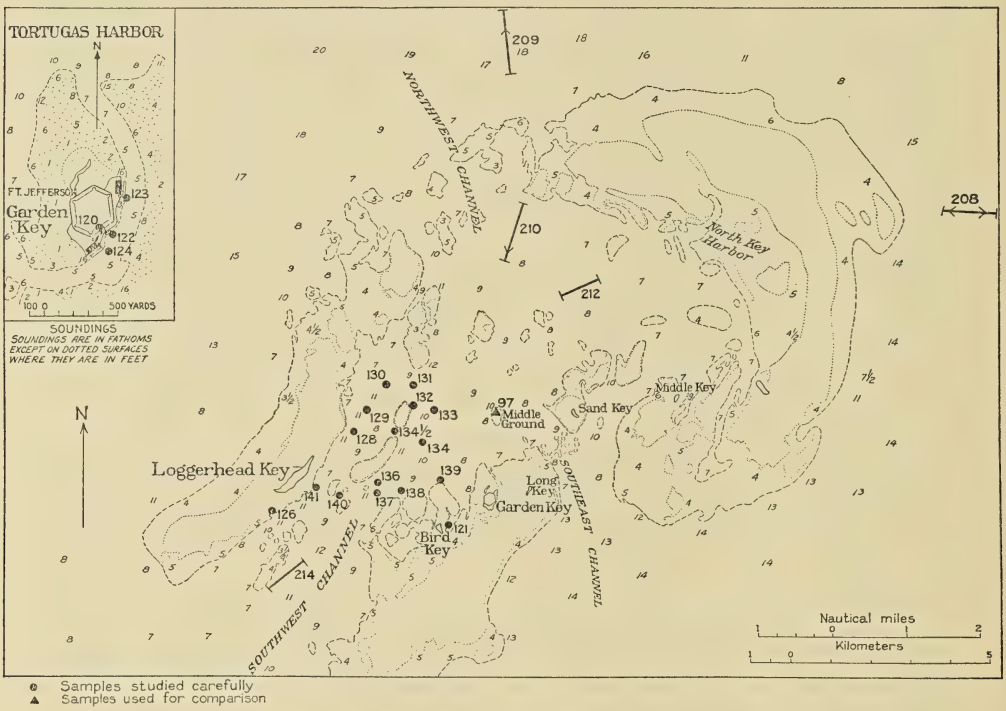


FIG. 1—Tortugas Harbor and approaches, showing location of bottom sample stations (Base, U. S. Coast and Geodetic Survey Chart No. 471a, issue February 1917).

The papers of Johnston and Williamson (1916b), Johnston, Merwin, and Williamson (1916a), and Wells (1917) dealt with calcium and carbonate equilibria in sea-water in connection with the solution and precipitation of calcium carbonate. These are again mentioned later in this report.

Interest in the calcium carbonate problem was again revived in 1928 by Vaughan, who had in the meantime taken over the directorship of the Scripps Institution. In 1928, Gee was added to the staff of that place as bacteriologist. Gee arranged experiments with Moberg, the chemist of the Institution, and he also received cooperation from Greenberg, associate professor of biochemistry in the University of California. These workers have

published various papers, both singly and jointly, since then. More will be said about these and other investigations in the subsequent discussion of calcium carbonate equilibrium.

QUANTITY OF MATERIAL STUDIED

The total number of bottom samples collected by Vaughan and his associates during the intervals between 1908 and 1915 numbered almost 300. About half of this collection was used by Vaughan, Matson, Goldman and others who obtained, and subsequently published, many new conclusions about the Floridian-Bahamian region. There remained, however, a large amount of material which had not been used. Furthermore, it represented as a whole good cross-sections of reef, lagoonal and beach sediments which could be compared easily with samples which had been described previously.

Specifically, 74 well-labeled samples were placed at the disposal of the writer for study. Several factors entered into his desire for studying them: First, the generosity of the collector, Dr. Vaughan, in permitting unconditional use of the material: Second, all of the samples had been analyzed in the United States Bureau of Soils Laboratory and, in addition, there were available several supplementary reports on them, such as one on diatoms by Dr. Mann, and another on the calcareous Algæ by Dr. Marshall A. Howe. Third, personal conference with Dr. Vaughan was possible at all times throughout the study. Finally, a detailed examination of a large number of samples from this region had not been made previously and there was definite need for such research before several questions could be settled.

METHOD OF PROCEDURE

Before actual examination of the material, it was deemed necessary to choose some kind of procedure which would yield at least rough quantitative results and yet allow fairly complete examination of all 74 samples within a reasonable length of time. The happy medium appeared to be somewhere between Goldman's technique and that of Matson (1910). Both workers produced usable information, but the former used methods which were time-consuming and inapplicable to a large number of samples, while Matson's results were too qualitative and not specific enough to include all principal types of organisms. It must be said that Matson was the first to attempt such microscopic descriptions and in so doing laid a groundwork for the advance of others. Bramlette's (1926) manner of describing samples is probably the nearest approach to what was wanted for routine examinations, but it was modified somewhat by the writer to fit the need of the investigation in hand.

A binocular microscope with low-power objectives (24 mm., 32 mm., 48 mm.), a good petrographic microscope, and a small hand lens constituted the optical equipment used. Grains having diameters between 2.0 and 0.05 mm.

were estimated and studied under reflected light by means of a binocular microscope; silt (0.05-0.005 mm.) and clay (0.005-0 mm.) particles were immersed in an index oil (N-1.515) on a glass slide and viewed by transmitted light. The hand lens was used on the bulk samples to check any irregularities in sampling or fractional splitting. Especially interesting objects were often sectioned or submitted to simple chemical tests. Histograms of all mechanical analyses were plotted for information on sorting. In addition, histograms of 76 samples described by others were plotted for comparison. Most of these latter samples had never been plotted previously.

DISCUSSION OF METHODS OF EXAMINATION

A bottom sample which has been analysed by the Bureau of Soils is screened into five grades having limits of 2-1 mm., 1-0.5 mm., 0.5-0.25 mm., 0.25-0.1 mm., and 0.1-0.05 mm. The remaining smaller particles are divided by settling methods into two grades called silt and clay, whose limits are 0.05-0.005 mm. and 0.005-0 mm., respectively.

It will be observed that a geometric ratio is roughly followed until sizes below 0.05 mm. are recorded. The jump between 0.05 and 0.005 mm. is a practical division based on settling velocities, but from a geometric standpoint it represents approximately three grades of particles. Likewise, if we adopt 0.001 mm. as a practical upper size limit for colloidal particles, the clay grade would embrace two geometric divisions in addition to colloidal particles. Therefore, when considering the plotted histograms one should keep in mind that the following grades are embraced in the two terms:

Silt:	0.05 -0.025 mm.	Clay:	0.005 -0.0025 mm.
	0.025-0.01		0.0025 -0.001
	0.01 -0.005	Colloid:	less than 0.001

Grains having a diameter of 0.05 mm. or less are extremely difficult to study, in fact it is not possible in most cases to make a dependable identification of a mineral. Nearly all of the shallow-water samples studied have the bulk of their grains lying between the limits of 2.0 and 0.05 mm.

Each grade is contained in a small homeopathic vial and the number of grains may vary anywhere from a few to several thousand. Obviously some uniform method of sample splitting was needed in order to fractionate a grade when the quantity of grains in it was large. A surgical scalpel having a blade length of 1 cm. and a width of 0.4 cm. was pointed to an arrow-tip shape. After mixing thoroughly the contents of a vial, this scalpel was introduced inside along the bottom of the grain pile for the length of the container. The grains which were retained when the scalpel was withdrawn were transferred to a small watch-glass for study. The procedure was repeated until about 300 grains were removed. If the vial contained only about 300 grains or less, the entire quantity was poured into the watch-

glass. The bulk of the sample which remained after sampling was always scanned under the low-power hand lens in order to be sure that the sampling was adequate and representative.

Dryden (1931) concluded that 300 grains represented the optimum number for a quantitative study of heavy mineral grains. With this number the error of counting is about 2 per cent. He also observed that the accuracy of count increased as the square root of the number of grains counted. The use of more than two significant figures is not justified, even if the count should reach as high as 4000.

Dryden's assumptions hold if the probability of drawing a particular kind of grain is $1/n_1+n_2+n_3+n_n$. Otto (1933) believes that conditions of random sampling are not likely to prevail even approximately in most methods. He enumerates four factors of divergence, namely, differences of size, shape, density and magnetic properties of grains. Thus magnetic grains tend to stick together; heavy grains sink rapidly through a mixture on slight vibration; grains having a low coefficient of friction slip away from a spatula more readily than those having a high coefficient. He also shows that large errors may arise when certain combinations of divergent characters are found. However, in a table given by him for determinations by spatula sampling, the mean average deviation for 21 species representing a count of 3012 grains was 5.8 per cent. The 21 species were arranged in groups of threes, and the average maximum deviation by groups was as follows: 1.9, 5.2, 6.0, 3.7, 5.3, 15.5 and 2.5 per cent. Examination of the group showing largest error (No. 6) revealed that it was made up as follows: pyrite, 382 grains; grossularite, 171 grains; and quartz, 71 grains; total 624 grains. Otto rightly points out that here the principal disturbing factor is a wide difference in densities with heavy grains being in predominance. Further examination of Otto's tables indicates that grains of uniform size, shape and density show a much lower error in count. It is evident that an attempt was being made to ascertain the more refined limits of sampling methods and that an error of nearly 6 per cent for the spatula method cast some doubt on its usefulness. Nevertheless, such a manner of sampling is extremely useful when the number of samples is large and time is an important factor. In this study 74 samples were represented by nearly 300 vials, and the average vial contained between 500 and 1500 grains. It was desired to segregate about 300 grains for each observation and the spatula method appeared to be far more rapid and less destructive to delicate organic grains than any other procedure yet suggested.

Even an indicated average mean deviation of about 6 per cent is not large when one considers errors incurred in collecting, handling and mechanically analyzing the sample. It is always desirable to have all of the errors of about the same magnitude in order to take full advantage of compensation, and the error of sampling in this study is believed to be as small, if not

smaller than any others involved here. Mechanical grading assures that all the grains contained in each vial are reasonably uniform in size. The nature of the material is such that there is little variation in average density while magnetic grains are almost absent.

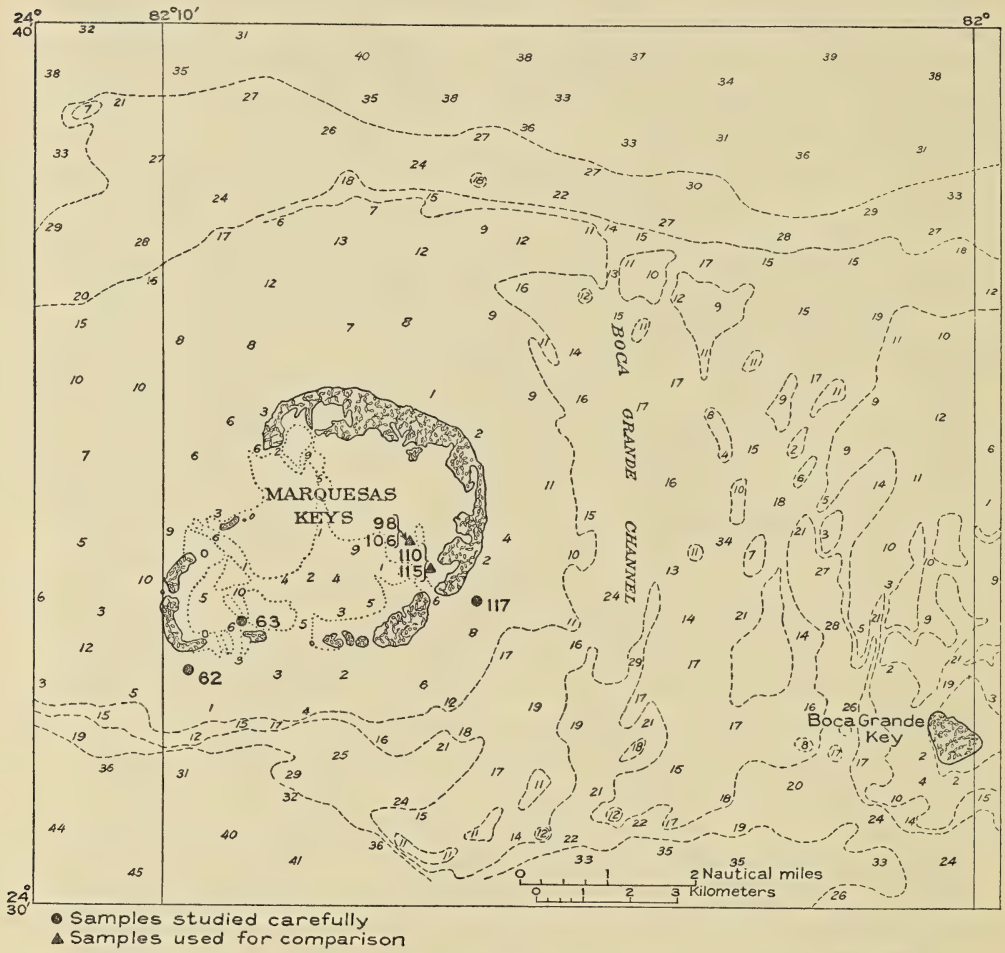


FIG. 2—Marquesas Lagoon, showing location of bottom sample stations (Base from U. S. Coast and Geodetic Survey chart No. 1252, July 1912).

The manner of arriving at a proper estimate is at best a personal accomplishment and the accuracy attained always contains some element of the personal equation. However, it is believed that almost anybody can become proficient enough to estimate within 10 per cent. The matter is simplified if the grains are of uniform size and color. Probably the worst way of doing it is to use too many grains at one time. About 300 is the maximum number that should be attempted at magnifications between 20 and 40 diameters. The microscope stage should be easily movable in all directions and in addition it should tilt readily. A 70-mm. watch-glass was used in connection

with a binocular microscope for observing all grains having a diameter greater than 0.05 mm. As nearly all of the material studied was white, it was necessary to paint the lower convex surface of the watch-glass with black paint.

Preliminary counting was done by the writer (Thorp, 1931) when he studied some deep-sea bottom samples from the Atlantic. At first the grains were counted in fields of approximately 100, but this number has been increased to 300. About 110,000 grains were seen and estimated during the course of study of the 74 samples. In the author's previous work, about 16,000 additional grains were observed.

A series of about 50 estimates was checked by actual countings and the error was found not to exceed 5 per cent in practically any case, the error often being surprisingly small. It is believed that the method of estimating is, after a little practice, nearly as accurate as actual count. Special care must be exercised when there are several species present in small but significant numbers. Dryden (1931) shows that the probable error of the abundant species will be much lower than that of the rare ones.

REVIEW OF THE GEOLOGIC HISTORY OF THE REGIONS

GEOLOGIC HISTORY OF THE FLORIDIAN PLATEAU

The following sketch was compiled largely from a report on the geology of Florida by C. Wythe Cooke and Stuart Mossom (1929).

That great projection of the North American continent which separates the deep water of the Gulf of Mexico from the deep water of the Atlantic Ocean is generally designated as the Floridian Plateau. Thus defined, the Plateau includes, in addition to the State of Florida, an equally great or a greater area that lies submerged in depths not exceeding 300 feet of water. Most of the peninsula lies east of the Plateau axis which trends about N 15° W.

Evidence that the Plateau probably existed as part of the ancient land mass of Appalachia is obtained from metamorphic rocks found in deep well borings from Marion County. During the Triassic and Jurassic periods and the Lower Cretaceous epoch the land probably was above sea-level. This evidence lies in well cuttings and consists of a series of limy, arenaceous and argillaceous sediments which aggregate 4000 feet or more in thickness.

During part of Upper Cretaceous time it was covered by the sea. During the Cenozoic era considerable oscillation of the shore line occurred, but the seas were always shallow and apparently the land was never elevated very high above sea-level.

Since the Piedmont Plateau, which was the original source of all the sand, gravel and clay deposited in rocks of the southeastern part of the Coastal Plain, is considerably removed from Florida, the sediments deposited on the Plateau are generally more calcareous than arenaceous or argillaceous.

The Eocene and part of the Oligocene shore line lay near the present edge of the Piedmont Plateau and the sediments deposited on the Plateau were nearly pure limestone. The Miocene epoch saw an influx of sand, mostly fine grained, which was transported southward to become intermixed with limestone. In later epochs much sand and clay from the Coastal Plain of Alabama, Georgia and the Carolinas migrated to the northern part of the Florida Plateau.

The structural features of the Plateau are simple. Little is known about the mica schists and quartzites which have been encountered in only one deep well. The sediments which overlie this basement are little deformed and occur in about the same attitude in which they were deposited. A gentle doming of the Eocene Ocala limestone occurred in central Florida. Central Florida appears to have been above sea-level in early and late, and possibly middle, Miocene. During part of early Miocene it was dry land, but late Miocene witnessed a submergence. Since Miocene the central and axial area of Florida has stood above sea-level, with considerable increase in altitude, but on the flanks of the dome there have been oscillations in the position of the shore line. Along the Florida reef tract the downward movement has amounted to several hundred feet.

GEOLOGIC HISTORY OF THE FLORIDA REEF TRACT

The summary which follows is based on papers by Vaughan (1914b) and Vaughan and Shaw (1915).

It is assumed that the reef tract participated in the uplift following Pliocene deposition over the previously submerged Florida Plateau. The magnitude of this upward movement was possibly 50 feet above present level for the key region.

The character of the present coast line shows the last important movement of the Peninsula to have been downward and that the keys even at Tortugas took part in the uplift and subsequent depression that affected the mainland, as they once stood 30 feet higher than now. At Miami, the conditions, as interpreted by Vaughan and Shaw, show that from a depression below sea-level there was an uplift to above sea-level by stages which aggregated 55 feet. This was followed by a depression of 46 feet and finally a small elevation of 10 feet. The land surface still remained 36 feet lower than previously.

Traces of oscillations as shown by soundings indicate that the reef tract has stood more than 120 feet above its present level. There have been numerous intermediate stands and minor oscillations, but at present no definite period can be assigned to them.

GEOLOGIC HISTORY OF THE BAHAMAS

The following sketch on the geology of the Bahamas is also taken from papers by Vaughan (1914a, 1919b).

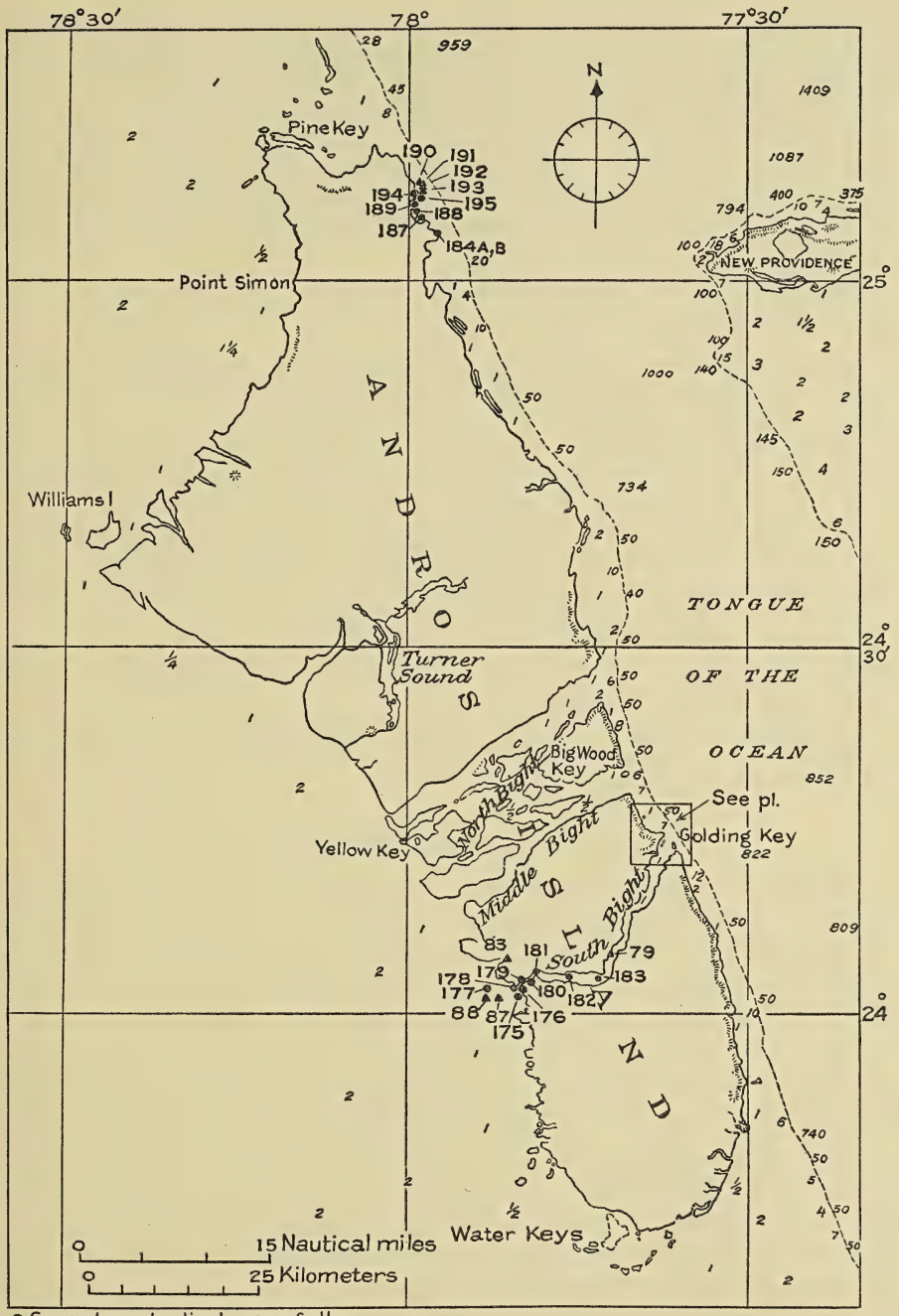


FIG. 3—Andros Island, Bahamas, showing position of bottom sample stations and detailed chart of Golding Cay entrance. (Base from Chart No. 26a, Hydrographic Office, U. S. Navy, 47th ed., December 1932.)

"The general country rock of the Bahamas is oolite. The sea-bottom from Gun Key to Northwest Passage, New Providence Island and Andros Island, is all or mostly composed of oolite, precisely similar to that of Southern Florida. The material forming the ridge at Nassau has been wind blown and is evidently a dune accumulation. Many or all of the ridges rising higher than 20 or 30 feet above sea-level have been formed of wind-blown material, of which oolite is an important constituent, but the oolite of most or all of the lower areas is of marine origin and has not been subjected to æolian action."

Vaughan's further account, changed to accord with the results of later investigations, is as follows:

(1) During Pleistocene time, perhaps earlier Pleistocene, numerous shallow submarine banks existed, on which calcium carbonate was chemically precipitated and which were covered by oolite.

(2) This submergence was followed by an uplift with the production by marine erosion of a platform and a margining shore cliff, and the formation of shore dunes paralleling the windward sea-front.

(3) Apparently there was further uplift and pot-holes were subaerially formed by solution on the platform between the dunes and the sea-front. At this time, the land on the east side of Andros Island stood at least 192 feet higher than now. (Vaughan, 1919b, p. 292.)

(4) This event was followed by submergence equal to or somewhat more than the previous uplift, and the present barrier reef developed on the offshore margin of the submerged platform.

(5) During Recent time there have been differential movements of minor magnitude, as is shown by the sea-cut caves elevated about 18 feet at Nicollstown Light, Andros Island.

COUNTERCURRENT, TIDES, AND PREVAILING WINDS

As has been suggested previously, waves and currents are of paramount importance in sorting the sediments exposed to their action. The Gulf Stream, although indirectly important to sedimentation, is less effective on the Florida coast than the southward flowing countercurrent. The movement of this body of water is according to the principles set forth by Bjerknes and according to the principles of alongshore wind-induced currents as enunciated by V. W. Ekman. The less dense fresh water flowing from the land spreads over the denser sea-water which lies to the east. Because of the effect of the rotation of the earth, this water is deflected southward counter to the Gulf Stream and close to the coast. This tendency is reenforced by the prevalent winds which in this region are predominantly from the east. The southward flowing inshore current follows the east coast of the United States from Cape Hatteras down through the Straits of Florida and then westward as far as Tortugas. Under normal weather conditions it extends sometimes as far as 11 miles offshore, but generally it makes a westerly course to Sand Key within the reef axis, although it sometimes runs north or south between the keys and northeast around Tortugas.

Tides and tidal currents run east and west or north and south across the keys. (For details, see Vaughan, Appendix 2 to this paper.) Often the bottoms of the passages are swept clean of sediments. Sometimes small keys are obliterated and others sometimes appear for an indefinite period due to the vagaries of currents and tidal action.

Southern Florida lies mainly within the Trade Wind zone, and winds blowing from the east predominate greatly over those coming from other

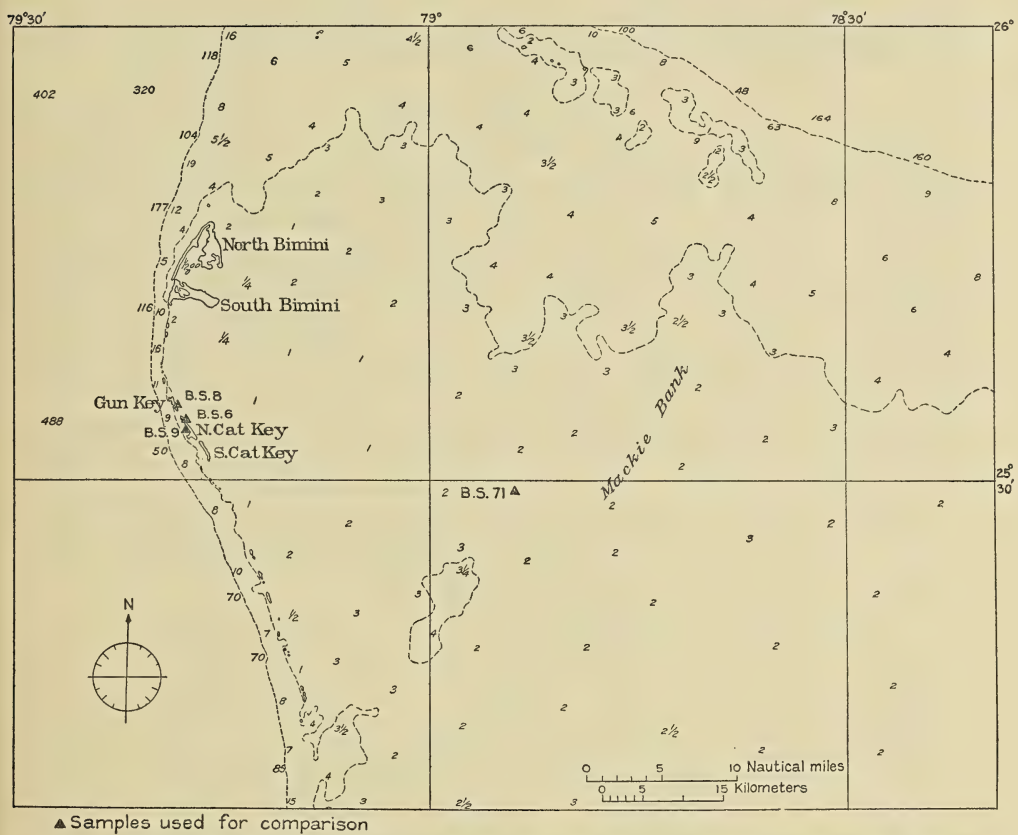


FIG. 4—Portion of the Great Bahama Banks, showing position of bottom sample stations. (Base, from Chart No. 26a, Hydrographic Office, U. S. Navy, 47th ed., December 1932.)

directions. This is well illustrated by wind rosettes for Key West (Cox, 1902). The Florida mainland configuration together with the outer boundary maintained by the Gulf Stream would confine an in-shore current driven by an east wind to a path paralleling the curve of the Florida Reef axis. Thus the formation of keys along such an axis is what would normally be expected.

Captain Hunt suggested, according to Vaughan (1910, p. 141), that the tides and wind tend to carry material from the reefs and flats to the area behind the keys; while the countercurrent moves material southward and

westward. The winds and tides bring material within the influence of the countercurrent by agitation of bottom sediment and are thus important in causing southward transportation.

The mechanics of marine erosion as applied to the east Florida coast are well described by D. W. Johnson (1918).

On the west coast of Florida there is an absence of along-shore currents and the coast line is minutely irregular.

ORGANISMS CONTRIBUTING TO THE BOTTOM DEPOSITS AND RECOGNITION OF THEIR FRAGMENTAL SKELETONS

CALCAREOUS ALGÆ

The most abundant organic constituent of the Florida and Bahamas bottom deposits is calcareous algæ, both Corallinaceæ and the genus *Halimeda* being common. They are present in all regions studied. Quantitatively, the corallines occur nearly twice as often as *Halimeda,* although on the northern part of the Florida reef tract the reverse is true. The corallines are occasionally very difficult to identify when present as small grains. Recognizable cells are rarely seen but the massive, relatively coarse, dense granularity is fully as diagnostic. Stem forms are usually preserved, even under intense erosion. Two other things which are likely to be confused with the corallines are broken worm tubes and coral fragments. The former are usually finer grained, while coral is lighter in shade and is inclined to be slightly translucent. In *Halimeda* the porous outer surface is preserved in nearly every case and identification is practically never difficult. The persistence of its cell-pore outlines is remarkable.

TABLE 1—*Order of quantitative importance for each constituent or aggregation.*

Florida		Bahamas		Florida and Bahamas	
Average of 50 samples	Per cent	Average of 24 samples	Per cent	Mean average of 74 samples	Per cent
1. Algæ, calcar.	25.1	Algæ, calcar.	18.0	Algæ, calcar.	22.8
2. Mollusk	17.5	Foraminifera	17.3	Mollusk	15.8
3. Silt	13.9	Clay	14.8	Silt	13.2
4. Coral	9.3	Mollusks	12.2	Foraminifera	11.7
5. Foraminifera	9.0	Silt	11.7	Clay	10.2
6. Clay	7.8	Coral	8.2	Coral	9.0
7. $CaCO_3$	5.3	$CaCO_3$	6.0	$CaCO_3$	5.5
8. Spicules, total	4.3	Pellets	3.8	Spicules, total	3.6
9. Minerals	3.9	Worm tubes	3.0	Minerals	2.8
10. Crustacea	1.4	Spicules, total	2.1	Worm tubes	1.8
11. Worm tubes	1.4	Oolites	1.6	Pellets	1.3
12. Bryozoa	0.4	Aggregates	0.8	Crustacea	1.2
13. Oolites	0.4	Crustacea	0.7	Oolites	0.8
14. Pellets	tr.	Minerals	0.5	Bryozoa	0.3
15.		Bryozoa	tr.	Aggregates	0.2
Total	99.7		100.7		100.2

Dr. M. A. Howe identified some specimens of calcareous algæ collected by Vaughan from localities at Tortugas, Andros Island, and off Key West. His list follows:

Report on Calcareous Algæ from Florida and the Bahamas, by Marshall A. Howe.

C-34 Andros Island, Bahamas. The calcareous incrustation belongs chiefly to the animal kingdom; a little of a young *Goniolithon* (?) on one piece.
C-35 Andros Island, Bahamas. Two specimens which I have designated as a and b.
C-35a *Goniolithon strictum* Fosl.
C-35b *Lithothamnium æmulans* Fosl. & Howe and young *Goniolithon strictum.*
C-47 Tortugas, Florida. Two specimens which I have distinguished as a and b.
C-47a *Lithothamnium æmulans* Fosl. & Howe.
C-47b Belongs to the animal kingdom (the smooth main crust).
C-48 Tortugas, Florida. Includes two specimens which I have distinguished as a and b.
C-48a *Lithothamnium incertum.* Previously known from Bermuda only.
C-48b *Lithothamnium æmulans* Fosl. & Howe.

Florida Reef Tract, T. W. Vaughan. Includes three specimens which I have distinguished as a, b, and c.

a. *Goniolithon propinquum* Fosl.
b. *Goniolithon borgesenii* Fosl.
c. *Lithothamnium syntrophicum* Fosl.

Pourtalès Plateau, 82 fathoms off Key West, Florida, J. B. Henderson. If these specimens bear any calcareous algæ, they are old, water-worn, and not identifiable. A thin white crust, with small elevated pores and having crowded globose-stelliform spicules, appears to belong to the sponge phylum. Various minute filamentous boring algæ, such as *Gomontia polyrhiza* (Lagerh.) Born & Flah., and *Ostreobium queketti* Born & Flah., are present in the calcareous mass and are microscopically conspicuous after decalcification.

TABLE 2

A. *Samples in which silt or clay exceeds 50 per cent of total weight.*

Region	No.	Silt	Clay
		p. ct.	p. ct.
Florida	121	56.2	...
Bahamas	180	...	56.4

TABLE 2—(*Continued*)

B. *Samples in which silt and clay exceed 50 per cent of total weight.*

Region	No.	Silt	Clay	Total
		p. ct.	p. ct.	p. ct.
Florida	129	37.9	15.0	52.9
	209	47.3	9.2	56.5
Bahamas	175	32.0	29.3	61.3
	176b	30.2	35.3	65.5
	177	31.1	40.1	71.2
	178	24.1	31.4	55.5
	179	31.1	40.2	71.3
	181	27.6	34.8	62.4
	183	24.9	37.5	62.4

C. *Samples in which one constituent equals or exceeds 40 per cent of total weight.*

Region	No.	Algæ, calcar.	Minerals	Total
		p. ct.	p. ct.	p. ct.
Florida	117	47.6	...	47.6
	198	...	47.2	47.2

D. *Samples containing 30 per cent or more and less than 40 per cent of a single constituent.*

Region	No.	Algæ. calcar.	Coral	Foraminifera	Mollusk	Minerals
		p. ct.	p. ct.	p. ct.	p. ct.	p. ct.
Florida	49	37.6	...	...	...	...
	50	33.5	...	...	31.1	...
	51	32.6	...	...	...	...
	52	37.6	...	...	...	...
	53	32.6	...	...	33.0	...
	55	34.8	...	...	32.9	...
	56	39.0	...	...	...	...
	57	36.9	...	...	...	...
	58	38.8	...	...	...	...
	59	34.4	...	...	...	...
	62	38.4	...	...	...	...
	120	31.4	...	...	...	...
	126	39.2	...	...	...	...
	199	...	...	...	...	39.0
	200	39.4	...	...	...	...
	206	37.7	...	...	...	...
	208	...	...	...	33.4	...
Bahamas	145	...	...	31.4	...	...
	146	...	...	32.0	...	...
	147	33.0	...	...	...	...
	159	30.0	...	...	...	...
	172	...	31.7	...	...	...
	187	36.4	...	...	...	...
	188	34.4	...	...	...	...
	189	38.2	...	...	...	...

E. *Maximum values for minor constituents.*

Sample No.	Crustacea p. ct.	Oolites p. ct.	Pellets p. ct.	Spicules p. ct.	Worm tubes p. ct.
63	4.0	...	...	...	...
134½	4.4	...	...	...	...
178	...	12.2	...	...	...
182	...	10.7	...	...	...
176a	...	...	29.5	...	...
183	...	...	20.3	...	...
212	...	...	...	11.9	...
213	...	...	...	10.4	...
144	...	...	...	...	6.5
145	...	...	...	...	10.5

A table giving chemical composition of the skeletons of marine algæ and marine invertebrates compiled from data given by Clarke and Wheeler (1922), Goldman (1918), and Bramlette (1926) is as follows:

TABLE 3—*Partial chemical composition of some organic constituents.*

	$CaCO_3$	$MgCO_3$	$CaSO_4$	$Ca_3P_2O_8$
Algæ, calcareous	89.50	9.75	0.75	.....
Coralline	80.00	19.00	1.00	.....
Halimeda	99.00	0.50	0.50	.....
Bryozoa	95.00	5.00	.....	.....
Coral	99.30	0.70	.....	.....
Crustacea	77.00	12.50	1.25	8.75
(Malacostraca and Ostracods)	.....	.....	.....	.....
Foraminifera, approximately	89.50	10.50	.....	.....
Amphistegina	95.20	4.80	.....	.....
Archaias	88.76	11.22	.....	.....
Quinqueloculina	90.11	9.33	.....	–
Mollusk	99.75	0.25	.....	.....
Worm (*Serpula*)	91.00	8.00	.....	1.00
Spicules:				
Alcyonaria	87.00	11.46	.....	1.00
Echinoid	90.00	9.00	1.00	.....

The consistently high percentage of calcareous algæ is noteworthy. It ranges from a fraction of one per cent in sample No. 180 from South Bight to a maximum of 47.6 per cent in sample No. 117 from Marquesas, the average of 74 samples being 22.8 per cent. Because of massive structure the skeletal material generally persists through one, and it appears likely, through more than one erosional cycle.

In many marine plants calcium carbonate is precipitated during synthesis as an incrustation over part or all of the surfaces of the organism. In the corallines and in *Halimeda,* calcium carbonate is precipitated within the cells and cell walls. The precipitate is formed when carbon dioxide is extracted from the water, thus reducing the content of bicarbonate ion and disturbing the equilibrium by increasing the calcium and carbonate ion product. Samples of incrusted eel grass, *Zostera,* suggestive of such organic precipitation, were collected at Tortugas by A. H. Gee, and a small similarly incrusted green plant, *Halophila,* collected in the Red Sea by Dr. Cyril

Crossland was shown to the writer. It should also be mentioned that carbonates precipitated by many living algæ, notably *Lithothamnium,* are relatively high in magnesium carbonate. This has been offered as an explanation for the typically dolomitic constitution of those formations high in algal material (Twenhofel, 1932, p. 308).

M. A. Howe (1912) suggests that coral reefs are largely composed of algal materials and that quantitatively these are of greater importance than are the corals themselves. This is borne out by study on the samples collected in Florida and the Bahamas. However, the importance of corals is not lessened by this fact, because they form the principal structure around which the algæ grow. Examination of geologically older reefs do not always show algal material to predominate over that of coral.

Reef sands, as might be expected, sometimes contain more algal remains than any other single constituent (Bramlette, 1926, pp. 6, 7). This also holds true for the Florida reef tract and the Bahama region.

MOLLUSKS

Second in order of abundance are the remains of mollusks, chiefly gastropods and pelecypods. The maximum quantity of molluscan shells is 33.4 per cent, observed in sample No. 208 from Tortugas, Florida. Other samples high in mollusks are Nos. 50, 53 and 55, collected from that portion of the Florida reef tract known as "the Quicksands." Like calcareous algæ, the mollusk shells are abundant, wide-spread and relatively refractory toward erosion.

In certain localities where there is little silt and clay, such as "the Quicksands" and the western part of Tortugas lagoon, these invertebrates thrive in greater numbers than in mud-choked areas like South Bight, Andros Island. In other parts of Florida, a limestone known as "worm rock" is now forming by accumulations of gastropod shells of the genus *Vermetus* (Matson, 1909).

Pteropods are generally open-sea pelagic animals and, therefore, are rarely encountered in these shallow water and near-shore deposits.

Recognition of mollusk fragments is not particularly difficult. External form, color, occasional translucency, nacreous linings, strong prismatic character and clear high birefringence are easily seen under the microscope.

Analyses of molluscan shells by Clarke and Wheeler (1922, pp. 38-39) show that they contain from 98 to 99.9 per cent of calcium carbonate.

FORAMINIFERA

Foraminifera contribute on the average about 12 per cent to the samples. The large and varied fauna has received consideration by Cushman (1922) and Norton (1930), the latter having made a critical study of the ecology and distribution of those species which occur in this area. For that reason, only the genera of those foraminifera which occur consistently or abun-

dantly are given. The nomenclature follows that set forth by Cushman in his classification (1933 a, b).

Most conspicuous of the genera is the large *Archaias*. This form is distributed over the entire region and for most samples comprises the bulk of all foraminifera present. *Peneroplis* and *Quinqueloculina* occur almost as frequently but not in such abundance. Certain forms, such as *Clavulina* and *Valvulina,* are more numerous in very shallow protected waters. Large thick tests are seen in the predominating groups of foraminifera. This is in line with observations on other marine plants and animals found here which secrete a calcareous body or cell structure.

A glance at table 3 will show that the tests of these protozoans contain about 90 per cent calcium carbonate and 10 per cent magnesium carbonates. Preservation of tests is excellent.

Occasionally such open sea pelagic forms as *Globigerinoides rubra* (d'Orbigny) and *Tretomphalus bulloides* (d'Orbigny) are found in shallow-water bottom deposits intermixed with shallow-water types. This is to be expected, as changing tides and currents, sweeping through passages between the keys, sometimes bring these pelagic foraminifera into shallow water. Norton (1930) in describing the micro-fauna of samples 84 and 85 from Tongue of the Ocean, off Andros Island, showed the reverse conditions—where shallow-water foraminifera, apparently after having been washed up on the beaches, were wind blown into deep water and intermingled with deep sea and pelagic types.

CORALS MADREPORARIAN

Since Ordovician time, reef corals have contributed to limestone formation in every part of the geologic column. A reef represents concentration of many organisms engaged in highly sustained biological activity. The volume of débris thus produced is considerable—far in excess of material removed by wave action. Coral reef investigations began early because it was the corals which first drew attention of naturalists to this region. The classical researches of Darwin, Dana, Murray, Agassiz, Semper, Davis, Daly, Vaughan, Andrews and others are too well known for further discussion here.

Mechanical abrasion, together with aqueous and organic transportation, produces muds and sands composed of material similar to that of the coral reef about which they lie. The deposits grade laterally and outward into other types, dependent on source of the incoming material, depth and currents.

Distribution of coral in the bottom deposits is somewhat localized, due chiefly to the fact that coral fragments appear to break down easily and, therefore, do not withstand transportation over even relatively short distances. This might offer some support to Murray's belief that the fine-grained oozes of the nearby deeper water were derived from comminuted coral fragments. However, a comparison of the quantity of living coral in

FIG. 5—Golding Cay entrance, Andros Island, Bahamas, showing location of bottom sample stations (plotted on a chart made by A. G. Mayor, 1912).

place with that of calcareous algæ and other large contributors will show that it is insufficient to account for the vast quantity of fine-grained ooze.

The highest value for coral (32.0 per cent) was found in Bahamas sample 146, obtained inside Golding Cay entrance. Sample 145, collected about

1000 feet away, was second highest. High figures were obtained in nearly all samples taken from protected areas such as Tortugas lagoon, Golding Cay entrance, and behind the reef at Cocoanut Point. This shows that the material collecting inside the lagoon is derived from nearby and most probably from the protecting reefs on the edge.

Madreporarian corals average about 99 per cent calcium carbonate and hence are important contributors to the formation of limestone.

Recognition of coral grains is somewhat difficult because there is little on which to base a determination. Large grains usually show sinuosities and trabecular structure, but these guides are not in evidence after a little mechanical or chemical erosion has occurred. The smaller grains show a semi-translucency which is fairly easy to distinguish from that of more translucent Alcyonarian spicule fragments. In making estimates, coral was generally ignored until all other constituents except calcium carbonate fragments were identified. Thus a few unrecognizable grains of coral were of necessity grouped with the latter.

SPICULES AND FRAGMENTS

Of the many objects encountered in these bottom samples, no one is more arresting in appearance than Alcyonarian spicules. Some species are colored a deep purple, while others are pinkish or colorless. The spicule proper is elongated, spindle shaped, and it exhibits a papillate surface which gives a slight frosty appearance to an otherwise transparent body. They occur throughout the bottom deposits examined, except in samples from South Bight, Andros Island. The preservation is very good; as a rule the spicules are entire.

Professor L. R. Cary (1918), who has conducted an exhaustive study at Tortugas on Alcyonarian corals and spicules, believes that these objects are underestimated in their importance as contributors of bottom material, since the bulk of calcareous matter composing the spicular skeleton of Gorgonians ranges from 20 to 35 per cent of the entire body weight of the animal. The abundance of Gorgonians suggests that they should contribute more calcium carbonate than do the stony corals. However, a count of spicules of the latter and of Madreporarian coral grains shows a ratio of about 1 to 2.5 in favor of the Madreporaria.

Chemical analyses of Alcyonarian spicules compiled by Professor Cary show that magnesium carbonate ranges from 11.5 to 16.9 per cent, calcium carbonate varies between 80.9 and 87.3 per cent, while calcium phosphate makes up from 0.12 to 0.44 per cent.

Sponge spicules, while not so abundant as Alcyonarian spicules, are almost as widely distributed. Identification is easy, as almost no structure is obliterated by erosion.

Echinoid spines, plates and fragments are not numerous enough to be of any special significance. Few or no difficulties are encountered in recognizing them.

Tunicate fragments and spicules are not believed to be particularly abundant, but one is likely to overlook them unless known material is examined beforehand in order to familiarize oneself with what to look for. Fragments often appear as smooth masses, having a fine spongy texture reminiscent of fine textured glassy pumice. The spicules, unfortunately, were not studied carefully enough or compared with other spicule-producing organisms, such as holothurians, to warrant accurate determinations. Some spicules obtained from a known tunicate were anchor shaped.

MINOR CONTRIBUTORS

Worm tubes are recognized by their external form and occasionally by the smooth cavity inside. Rarely one finds small crenulate jaws which are assignable to worms. A protected body of water, such as Golding Cay entrance, appears to be especially favorable to their growth. The east side of Tortugas lagoon is another place where worm remains are common. Broken and eroded tubes may not be identifiable and thus will be grouped with calcium carbonate fragments.

Both decapods and ostracods are constantly present throughout the bottom deposits, although the latter are about twice as common. The center of Tortugas lagoon seems to be somewhat more favorable than other places for the existence of ostracods, if one may judge by the relative commonness of their tests. Ostracod valves are almost always identifiable as such, but decapod fragments may present difficulties. Usually a fine prismatic structure and fibrousness will be discerned in the latter.

Bryozoa rarely are abundant enough to be considered of importance, but they do occur fairly well distributed over the region and are relatively common in Tortugas lagoon.

Quantitatively, diatoms are unimportant in the bottom deposits examined. It is thought that some of them may be lost in the process of mechanical analysis. In 1914, Dr. Vaughan submitted 24 Florida-Bahama samples to Dr. Mann for study of the diatoms. The complete report is included as an appendix to this paper.

UNDIFFERENTIATED CALCIUM CARBONATE FRAGMENTS

This classification was adopted to take care of those undetermined grains which were essentially composed of calcium carbonate and which appeared to be of organic origin, yet it was not safe to place them in any definite organic category for lack of characteristic features. A few fragments of compact limestone are included. Observation leads the writer to believe that of the grains thus segregated, the things which are most likely to be represented are, in order of importance: (1) coralline algæ, (2) coral, (3) worn tubes.

If the quantities were of a greater order of magnitude, it might be of value to make additional careful examinations, but it is felt that the amount transferred by closer identifications would cause no appreciable change in the general interpretation.

PELLETS

In the 15 samples listed in table 4, there were observed a number of elongate ellipsoidal bodies which were composed of aggregated fine material, similar to the unaggregated silt and clay of the same bottom sample, and which ranged in size from 0.2 to nearly 2 mm. in their longest axial diameter.

TABLE 4—*Distribution and percentage of pellets.*

Sample No.	Locality	Depth (feet)	Percentage of pellets
	A. Florida:		
63	S.W. corner Marquesas lagoon	2	2.0
131	S.W. part of Tortugas lagoon	about 66	2.8
143	Featherhead Bank, Biscayne Bay	2	0.6
205	Off Rodriguez Key	12	0.1
209	Outside Tortugas lagoon on north (dredged)	114–120	3.0
	B. Bahamas:		
147	South Bight, east entrance	about 7 ?	tr.
175	South Bight, west entrance	about 2	1.0
176a	South Bight, west entrance (shore material)	0	3.6
176b	South Bight, west entrance (shore material)	0	29.5
177	2 Miles West of mouth of South Bight, west entrance	6	13.0
178	South Bight, west bank of west end entrance	2	0.9
179	South Bight, S.E. bank on west end of South Bight canal (shore material)	0	6.8
180	South Bight, a little east of No. 179 (shore material)	0	7.5
181	South Bight, bar at head of South Bight canal	0 ?	8.0
183	Inlet north of South Bight	0 ?	20.3

The texture is somewhat loose and the soft friable nature is shown when they are easily broken by a needle point. All specimens were opaque and white, except that occasionally the color varied toward neutral gray. Photomicrographs published by Vaughan (1924) reveal no concentric structure, but the presence of a darker region on the outside of the pellets indicates what Moore (1933) suggests as being one of "slightly finer material, possibly, to some extent, cemented with mucus or some other material." If this is the case, they are similar in structure to the pellets described from the Clyde Sea (Moore, 1931, 1931a; Galliher, 1932, fig. 2). Also the darker area may represent the reduced state of an infinitesimal amount of iron contained organically and reduced by bacteria which attack the mucous coating of the pellet.

Again referring to table 4 it is noticed that except for five scattered samples showing relatively few pellets, the greatest bulk of specimens was obtained in samples from around and in South Bight, Andros Island. Highest figures were obtained for the west portion, there being only a trace in sample 147 from the east entrance. Shore material yielded the largest numbers, therefore it is indicated that the pellets originate in the littoral zone and are transported by tide, waves and currents from that region into the delta, estuary and marginal lagoon deposits.

These same ellipsoidal bodies had been observed in the muds of South Bight as early as 1912 by Vaughan, who at that time believed them to represent early stages in the formation of oolites. In 1924 this opinion was modified (Vaughan, 1924, p. 327) by the statement that the earlier experiments were too rudely conducted to support such a contention. Since the size, form and general structure of the pellets resembled glauconite and greenalite grains, he said that when the formation of the latter two minerals is explained it would probably have wide application.

As early as 1890 Buchanan had described elongated pellets from some mud collected off Arran in the Firth of Clyde. From the Gulf of Guinea, Philippi (1908) described and figured glauconitized pellets which he thought were echinoderm feces. Takahashi and Yagi (1929) found elongated ellipsoid grains in bottom samples collected in several localities around Japan. These grains, or coprolites, as they were tentatively identified, ranged in color from gray to green. A summary of the paper is given as follows:

"1. Peculiar mud-grains are found widely distributed in the estuarine and littoral regions.

"2. They are supposed to be the excrements of certain mud-eaters, and tentatively designated as coprolite.

"3. They were separated and investigated both physically and chemically with the result that they were found to become gradually glauconized when the conditions are favorable. Such grains are termed copro-glauconite.

"4. There is found every stage from plain coprolite-grain to glauconite; at first the pigmental glauconite appearing in patches inside of the grains, which gradually broaden their areas until the whole grains become glauconized.

"5. They were compared with, and positively identified with the fossil glauconite.

"6. The origin of certain glauconite in the littoral deposits of the geological ages thus suggested itself to be, in part at least, fossil coprolites glauconized."

The writer (1931) described some coprolite-bearing muds from off the eastern coast of Panama, although some doubt at that time was expressed about their fecal origin. The dark-brown or olive-drab color, size and other characteristics accord with those described by Takahashi and Yagi.

Another method of attack on the question of origin has been furnished by the studies of Dr. H. B. Moore on fecal excrement of marine invertebrates. This investigator has collected and described fecal material from muds of the Clyde Sea area as well as from known species confined to aquaria. Fecal pellets thus described are slightly smaller than those observed here, but there is a great deal of similarity in general shape and structure. Of the organisms described, the marine gastropod *Littorina* and some Maldanid worms yield feces most like the South Bight objects. However, no special search has as yet been made for the presence of these two organisms in South Bight. Sections made on specimens from samples of South Bight material did not reveal a concentric structure, but there are often observed imbedded fragments of calcareous algæ, foraminifera and various pieces of débris. These objects may not necessarily represent the food of an animal but they might have been coated with bacterial slimes, available as foodstuff.

In concluding this discussion of ellipsoidal pellets, the writer wishes to state that he accepts the results of Moore on the fecal origin of some pellets, but he believes that the problem is not yet sufficiently clarified to include all soft bodies which bear close resemblances. There is a large area off eastern Panama, as mentioned, and another off the western coast of Mexico which will be described by R. R. Revelle in a forthcoming paper. Why these well-defined places should contain an abundance of pellets, almost to the exclusion of all other constituents, and why a much greater portion of the ocean floor appears to be lacking in recognizable pellets is still to be answered by future research.

ECOLOGY OF THE ORGANISMS

FAVORABLE AND UNFAVORABLE CONDITIONS

Something has already been said about ecologic relationships in the discussion of individual organisms. Conditions especially favorable to life are shown to exist within a lagoon because of the presence there of an abundance of relatively fresh uneroded material and representatives of many types of organisms. Conversely, a narrow mud-choked passage, such as South Bight, is relatively deficient in organic types with the possible exception of a few organisms such as mud feeders which have become specially adapted to the peculiar environment. This point is well illustrated by comparing the quantity and variety of organic constituents listed in table 13, c and f, for samples Nos. 175 to 183 from South Bight, and samples Nos. 184a to 195 from behind a reef on the northeastern coast of Andros Island.

Reef-living organisms thrive best when there is a constant interchange of water and general absence of silt and clay. Consistently high figures for the percentage of organic remains are recorded in the same table for samples Nos. 49 to 62, collected from that part of the Florida reef tract known as

"the Quicksands." The water is shallow and current action is vigorous enough to remove most of the silt and clay as rapidly as it forms.

Another factor which influences the distribution of organisms is the alkalinity or acidity of the water in which they live. Foraminifera supply examples of the formation of large, heavy tests in comparison with the quantity of protoplasm, under conditions of high alkalinity. In less alkaline types of water, such as occur in stagnant bays and at great depths, foraminifera often develop chitinous tests instead of normally calcareous ones. Species and even genera of foraminifera are sometimes useful in determining the relative depth of water above a bottom deposit. *Archaias, Peneroplis, Clavulina,* and a few massive *Quinqueloculina* typically dwell in very shallow water. To a lesser degree, *Asterigerina* and the large Rotaliidæ are characteristic of slightly greater depths, but usually not exceeding 150 feet.

The use of corals as depth indicators is well known and need not be reviewed here.

Vaughan (1933, p. 355) mentions that the presence of fossil nullipores, if they are *in situ,* indicates relatively shallow water because they are photosynthetic organisms. It is known that light does not penetrate to depths of more than a few hundred meters in sea-water. Knowledge as to the maximum depth to which nullipores will live has not yet been obtained.

TEMPERATURE AS A CONTROLLING FACTOR

Vaughan (1918, p. 321) has shown that winter temperatures limit extension of sea-level and near sea-level reefs on the northern part of the reef tract. However, there are circumstances under which considerable havoc may be wrought by high temperatures. Some very low tides expose large areas of normally submerged flora and fauna to the direct influence of the heat of the sun for a sufficient length of time to kill organisms. Desiccation also enters into the effect, but many organisms are able to withstand a considerable degree of drying. The literature on relationship of corals to temperature is voluminous.

NUTRIENT MATERIAL IN SEA-WATER AND ITS CONTROL OF ORGANISMS

About 30 years ago Brandt, in attempting to explain why the quantity of phytoplankton in the tropical waters seemed to be relatively small, suggested that it was because denitrifying bacteria reduced the nitrates to nitrites and ammonia. This suggestion caused Drew to look for denitrifying bacteria in the Bahamian and Floridian regions.

The abundance of phytoplankton appears to be closely related to the quantity of available nutrient materials in sea-water. Professor Moberg (1929) in a recent paper has summarized this relationship as follows:

"(1) The food of practically all marine animals is derived from the photosynthetic activity of pelagic marine plants, *i.e.* phytoplankton;

"(2) The growth of these plants is controlled by certain physical conditions and by the abundance of a number of chemical substances in solution in the water;

"(3) Some of these substances occur in the sea-water in exceedingly small quantities and during periods of rapid plant growth may entirely disappear;

"(4) These substances are restored to the photosynthetic zone chiefly by vertical circulation, the bottom water being rich in nutrient substances derived from the decomposition of organic material."

To these four facts he adds that in higher latitudes nutrient substances are restored by vertical mixing of the water which occurs when the surface water cools in the autumn and winter.

The most important nitrogen compounds in sea-water, as far as plankton is concerned, are the nitrates. Some algæ can use nitrites and ammonia but these two substances "are found in very minute quantities or less regularly" (Gran, 1931, p. 38). The amount of nitrate calculated as N is observed to vary between 0 and 700 mg. per cubic meter. Gran (1931) says that the quantity sufficient for a normal rich growth of diatoms on the coast of northern Europe may be 90 mg. per cubic meter. The quantity of phosphate, reckoned as P_2O_5, varies between 0 and 200 mg. per m^3, while the quantity present in the surface water of northern Europe, just before a rich growth begins in the spring, is about 40 mg. per cubic meter.

Gran (p. 38) also quotes other workers who found that the phosphate concentration decreases with the increase of pelagic diatoms, and that, after the phosphates at the surface are used up, the maximum of phytoplankton is followed by a minimum, the diatoms ceasing to propagate and sinking to the depths. Another worker, Schreiber (see Gran, 1931, p. 38), showed by culture experiments that the productivity of the North Sea water is limited by the lack of either nitrates or phosphates.

Wattenberg (1931) points out that although nitrates and phosphates are limiting factors in plankton production, they are not the only ones, since light and small quantities of iron compounds have been shown to be necessary for growth. In addition, there must be present the necessary test-forming materials, chiefly calcium salts and silica.

Atkins (Wattenberg, 1931) has observed during strong diatom growth almost entire depletion of dissolved silica. However, in normal sea-water silica is usually present in such quantity that the nitrates and phosphates are consumed before the silica threshold is reached. The sea-water off the coasts of Florida and the Bahamas may not be normal with respect to silica.

The abundance of marine life around the coasts of Florida and the Bahamas suggests that there must be quantities of smaller organisms which serve as food. Drew and Bavendamm showed the presence of many denitrifying bacteria, and Mann (see appendix) noted the scarceness and fragility of diatom frustules in the deposits, hence it appears that condi-

tions are not favorable for the growth of phytoplankton. Furthermore, the thin and poorly shaped diatom skeletons described by Mann are probably due, as Vaughan has suggested, to insufficient silica in the sea-water. This is probably caused by the absence of any nearby source of silica. Krogh (1931) has paid attention to the importance which dissolved organic substances may play in the nourishment of phytoplankton, but he has not considered the organic content of the bottom deposits. Trask (1932) has made determinations of the organic content of several hundred bottom samples from all parts of the world, including a few from these regions. A sample from Florida Bay showed about 2.4 per cent organic matter. Other samples (pp. 249-273) ranged from about 0.08 to 3.4 per cent. His average for organic content of near-shore sediments was approximately 3 or 3.5 per cent (Trask 1932, p. 21).

Trask used the following factors for obtaining values of organic matter and carbon from nitrogen determinations:

Organic Matter	$=14 \times N$
Carbon	=organic matter/1.7
C/N ratio	=8.4 (approximately)

It appears that these sediments, compared to other kinds of near-shore sediments, are slightly below average in organic content.

OOLITES

GENERAL DISCUSSION

Nothing found in the bottom samples of this region is more interesting than the indurated calcareous ellipsoidal bodies whose origin is still somewhat obscure.

The size of these oolites ranges from 0.5 to a maximum of about 1.5 mm. Normally, they are considerably more elongated than soft coprolitic pellets. The surfaces are smooth, almost porcellanous in some cases, and the texture is fine grained and compact. Thin sections reveal a concentric structure, sometimes grouped about a nucleus and sometimes with no distinguishable nucleus. Soft pellets show little or no concentric structure.

Vaughan was led to study them rather carefully in connection with his investigation of the Florida oolites. Since soft pellets of comparable size and shape often occurred together with the indurated oolite grains, he was inclined to view the former as representing the initial stage of oolite creation. Later, he (1924, p. 327) decided that the experiments on growth of oolite grains were not sufficiently accurate for proof, thereby leaving it an open question as to the origin of the two types. As in the case of coprolitic pellets, most oolite grains were seen in samples from west South Bight, Andros Island. Fifteen samples, distributed pretty well over both the Florida reef tract and the Bahamas, contained them, usually in small quantities.

Only rarely were glistening fresh oolites seen. Of the samples used for comparison, No. 71, collected on the Great Bahama Bank, is described by Vaughan (1918, p. 274) as being an oolitic sand. The oolites were derived from older rock. A partial chemical analysis (table 6) shows its composition to be nearly pure calcium carbonate (99.49 per cent). Analyses of three oolites (table 6) show similar composition for A and B, but a mixture of silica (presumably quartz) in sample B changes the relationship slightly.

TABLE 5—*Distribution of oolites.*

Florida		Bahamas	
Sample	Per cent	Sample	Per cent
No. 49	1.2	No. 146	0.2
50	1.4	159	1.3
51	3.0	175	1.1
53	1.0	176a	2.7
54	0.4	177	1.0
55	tr.	178	12.2
56	3.0	182	10.7
57	0.4	183	2.3
58	0.4	184a	1.6
59	0.6	184b	1.8
62	0.5	187	0.9
63	0.2	188	0.7
117	0.2	189	tr.
128	tr.	194	1.7
133	0.5	195	0.2
134	1.4		
134½	tr.		
136	1.0		
137	0.1		
138	tr.		
140	tr.		
141	tr.		
143	tr.		
198	1.0		
199	1.0		
200	0.4		
201	0.2		
204	1.2		
205	1.0		
208	0.8		

The oolites in the bottom deposits have probably been derived from the Pleistocene oolitic formations which stand above and extend below sea-level in the Bahamas, around the edge of Biscayne Bay and on keys of the reef tract from West Summerland Keys, near Bahia Honda, to and including Boca Grande Key. West of Boca Grande Key the formation is below sea-level, but borings show that it constitutes the basement of Marquesas lagoon. It is therefore probable that the few oolites found in the Marquesas and on the Quicksands are derived from submerged oolite. The

keys of the northern part of the reef tract from Soldiers Key southward and westward to Bahia Honda are not oolitic but are composed of elevated coral-reef limestone. Oolites of the Great Bahama Bank may also be derived from below. Quoting Vaughan (1914a, p. 50):

"The sea-bottom from Gun Key to Northwest passage, New Providence Island, and Andros Island is nearly all or mostly composed of oolites, precisely similar to that of southern Florida."

TABLE 6—*Chemical analyses of oolite and bottom samples from Florida and the Bahamas (by W. C. Wheeler).*[1]

	A Oolite Boca Grande Key, Florida	B Oolite Everglades, Miami, Florida	C Oolite Sharp Point, Andros Island	Bottom sample[2] (98) east side Marquesas Lagoon, Florida	Bottom sample[3] (87) 1 mile west of west end of South Bight, Bahamas
CHEMICAL ANALYSES					
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*
SiO_2	0.03	8.23[4]	0.07	1.13	0.28
Al_2O_3	.18	.00	.00	.14	.03
Fe_2O_3	.22	.21	.13	.21 (total Fe)	.11 (total Fe)
MgO	tr.	tr.	tr.	1.31	1.25
CaO	53.77	51.60	54.57	51.04	52.30
Na_2O	.90	.11	.14	.....	.....
K_2O	tr.	tr.	tr.	.....	.....
H_2O	1.21	.17	1.72	2.03 (and organic)	3.16 (and organic)
CO_2	42.34	40.11	43.07	41.50	42.45
P_2O_5	tr.	tr.	tr.	.....	.....
SO_3	.28	tr.	.14	.....	.....
Cl	1.02	.08	.03	.....	.....
Soluble	.....	.....	.....	2.21[5]	.....
Total	99.95	100.51	99.87	99.57	99.58
REDUCED ANALYSES (HYPOTHETICAL COMBINATIONS), H_2O, ORGANIC MATTER, AND SOLUBLE SALTS REJECTED; SILICA NOT ESSENTIAL.					
SiO_2	0.03	8.19	0.07	1.18	0.29
$(Al,Fe)_2O_3$	.42	.21	.13	.37	.15
$MgCO_3$	tr.	tr.	tr.	2.88	2.72
$CaCO_3$	99.05	91.60	99.56	95.57	96.84
$Ca_3P_2O_8$	tr.	tr.	tr.	.....	.....
$CaSO_4$	.50	tr.	.24	.....	.....
Total	100.00	100.00	100.00	100.00	100.00

[1] From Vaughan (1917, p. 937; 1918a, p. 269; 1924, p. 319.)
[2] Sample washed and dried over H_2SO_4.
[3] Sample filtered, washed and dried over H_2SO_4.
[4] 25 per cent soluble SiO_2; the rest of the silica appears to be white sand.
[5] Saline salts not washed out by water in the preparation of the sand.

THEORIES OF ORIGIN

Artificial oolites have been prepared by Vaughan (1914a, p. 229). Spheroids of $CaCO_3$ were also precipitated from sea-water by Kellerman

(1914, p. 400). Usually some kind of nucleus, such as calcite grains, aragonite needles, quartz grains or almost any minute particle will serve as a starting point for artificial oolites. Even small bubbles of gas have been suggested as possible nuclei.

Various theories have been proposed to account for natural oolite formations. Rothpletz (1892) decided that oolites of the Great Salt Lake were developed through the agency of algæ belonging to the genera *Glœocapsa and Glœotheca.* Similar oolites developing in some portions of the Red Sea were also believed formed by this means. Linck (1903) postulated that whenever the quantity of calcium carbonate in sea-water exceeds the limit of maximum solubility for the conditions, it is precipitated as calcium carbonate (calcite) in temperate, and as aragonite in tropical, latitudes. He considered the algal rods of Rothpletz as being minute crystals of aragonite.

Bucher (1918) has considered a colloidal origin as the probable explanation. To quote:

"—most if not all, oolitic and spherulitic grains were formed by at least one constituent substance changing from the emulsoid state to that of a solid; that the spherical shape of the grains is due to the tendency of the droplets formed during this process of separation to coalesce; and that the difference between radial and concentric structure depends on the amount of other substance thrown out simultaneously with, and mechanically enmeshed in, the growing structure."

Thus we have the organic theory of Rothpletz, the simple physicochemical precipitate of Linck and Vaughan, and the colloidal coalescent conditions described by Bucher. To this list must be added the explanation given by Mathews (1930) to account for the origin and growth of oolites in the Great Salt Lake.

The Salt Lake oolites first have their origin at the water's edge. From here they are washed up on the mud flat; and as the wind drives them inland laminæ are produced seasonally by the direct precipitation of aragonite from the evaporation of capillary water. Growth occurs only during early summer when rise of temperature is greatest and only little or no growth takes place in the water. The oolites found on the bottom are small and contain but one or two laminæ. That growth of laminæ occurred on land is shown by accumulation of soot on the outside of the crystalline laminæ, and each band of soot was gathered after the rainy season.

The elementary conditions of this theory are applicable to those around and in South Bight. No deposit of soot was seen between the laminæ nor is any other seasonal variation apparent. However, the Bahamian oolites do show many laminæ which suggests frequent interruption during the growth cycles.

A specimen of bottom mud collected by Dr. Vaughan from near Boca Chica Key, near Key West, was shown to the author shortly after the section on oolites was written. The many perfect oolite grains, which this

bottom mud contained, may have been recently formed. However, since the material was entangled in the roots of a mangrove tree, there is a chance that it was derived from the oolitic rock of which the key is composed. Until more material is collected and studied, it would not be safe to assume that the formation of oolite is in progress at that place, even though it is suggested by this one specimen.

CHEMICAL DETERMINATIONS OF CALCIUM, MAGNESIUM AND CARBONATE

Twenty samples representing a cross-section of the area were analyzed for calcium, magnesium and carbonate. Standard procedures, such as given by Engelder (1929, pp. 81, 216), were employed to determine calcium and magnesium, and carbonate was determined indirectly by measuring the

TABLE 7—*Partial chemical analyses of twenty representative samples.*

1 Sample No.	2 CO_3	3 Ca	4 Mg	5 Total	6 $CaCO_3$	7 $MgCO_3$	8 $CaCO_3$+ $MgCO_3$	9 Excess CO_3	10 Ca/Mg ratio
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	
51	55.17	33.80	0.51	89.48	84.40	1.78	86.18	3.30	66.3
54	55.63	36.80	0.51	92.94	91.90	1.78	93.68	−0.74	72.1
63	55.23	35.60	0.56	91.39	88.91	1.92	90.83	0.56	63.6
117	57.13	37.30	0.27	94.70	93.15	0.93	94.08	0.62	138.2
121	54.03	33.20	0.52	87.75	82.91	1.80	84.71	3.04	63.8
132	56.86	35.30	0.59	92.75	88.16	2.06	90.22	2.53	59.9
134	56.59	35.60	0.48	92.67	88.91	1.65	90.56	2.11	74.2
145	56.86	35.20	0.89	92.95	87.91	3.07	90.98	1.97	39.6
159	57.27	35.00	0.57	92.84	87.41	1.99	89.40	3.44	61.4
175	56.59	36.10	0.58	93.27	90.16	2.03	92.19	1.08	62.2
177	56.59	36.40	0.40	93.39	90.91	1.38	92.29	1.10	91.0
178	55.23	34.50	0.54	90.27	86.16	1.87	88.03	2.24	63.9
182	57.00	36.60	0.48	94.08	91.40	1.67	93.07	1.01	76.3
183	55.09	35.80	0.40	91.29	89.41	1.37	90.78	0.51	89.5
195	55.36	36.00	0.66	92.02	89.91	2.29	92.20	−0.18	54.5
199	23.73	14.30	0.13	38.16	35.71	0.44	36.15	2.01	110.0
204	55.50	36.20	0.42	92.12	90.41	1.45	91.86	0.26	86.0
205	56.86	36.80	0.40	94.06	91.90	1.40	93.30	0.76	92.0
206	57.00	36.70	0.37	94.07	91.65	1.30	92.95	1.12	99.2
212	57.00	35.70	0.64	93.34	89.16	2.23	91.39	1.95	55.7
Average	54.54	34.64	0.50	89.68	86.00	1.73	88.22	1.46	76.0

$CaCO_3 = Ca \times 2.4974$
$MgCO_3 = Mg \times 3.4673$
$Ca/Mg = 76$ (average)
$CaCO_3/MgCO_3 = \frac{Ca/Mg}{1.3884} = 54.74$ (average)

loss of weight caused by removal of carbon dioxide from the dried and weighed sample. For the method used to determine carbonate (Scott, W. W., 1925, pp. 1223-1225) the errors may range as high as 3.5 per cent, but in most cases it is less than 2 per cent. As a rule the CO_2 values tend to be too high rather than too low. Table 7 gives a summary of results.

The first six columns show, respectively, sample number, total CO_3 computed from the CO_2, total calcium measured, total magnesium measured, and finally, the combined total of carbonate, calcium and magnesium. In columns 6 and 7, calcium and magnesium are computed as their respective carbonates. Column number 8 shows the sum total of computed $CaCO_3$ and $MgCO_3$. Column number 9 shows the difference between the measured values of CO_3 combined with calcium and magnesium and the computed values of CO_3 in the $CaCO_3+MgCO_3$. The difference may also be expressed as excess or insufficient carbonate. The agreement is good. There seems to be little doubt that practically all the calcium and magnesium are combined as carbonates in the sediments.

TABLE 8—*Ratio of calcium to magnesium according to localities.*

Locality	Sample No.	Ca/Mg	Average
East Side, Andros Island	145	39.6	
	195	54.5	
	159	61.4	
			51.8
Tortugas	121	63.8	
	132	59.9	
	134	74.2	
	212	55.7	
			63.4
West Side, Andros Island	175	62.2	
	177	91.0	
	178	63.9	
			72.4
South Bight, Andros Island	182	76.3	
	183	89.5	
			82.9
Florida Reef, west of Pine Islands	51	66.3	
	54	72.1	
	63	63.6	
	117	138.2	
	205	92.0	
	206	99.2	
			88.7
Florida Reef, north of Barnes Sound	199	110.0	
	204	86.0	
			98.0
Regional average	.....	.....	76.0

Organic matter was not determined, nor were the samples ignited, hence, values for $CaCO_3$ and $MgCO_3$ are proportionately lower than for analyses of similar samples given by Wheeler in tables numbers 6 and 9.

The predominantly calcareous nature of these sediments is at once evident by looking at table 7. The maximum value for $CaCO_3$ is 93.15 per cent in the case of sample 117. When the samples are arranged into percentage groups, it is found that 40 per cent of the samples contain over 90

per cent calcium carbonate; 85 per cent, over 85 per cent; and only 5 per cent (1 sample) less than 80 per cent calcium carbonate. This one sample is interesting because it is the only one analyzed which contains an abundance of quartz. The average calcium carbonate percentage for the whole series is 86.00.

TABLE 9—*Partial chemical analyses of bottom samples from Florida and the Bahamas (by W. C. Wheeler).*[1]

No.	Locality	Analyses				Reduced analysis, hypothetical combinations			Remarks
		Insoluble	CaO	MgO	CO_2 Calc.	Insoluble	$MgCO_3$	$CaCO_3$	
		P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	
68	Beach sand from east side of Sands Key, Florida.	1.15	51.77[2]	1.73	42.55	1.11	3.73	95.08	As received.
71	Great Bahama Bank between Gun Cay Light and Northwest Passage, Bahamas.	0.13	53.98	0.18	42.42	0.13	0.38	99.49	An oolite.
79	South Bight, Andros Island, Bahamas.	0.46	51.75	1.16	42.94	0.47	2.56	93.36	Washed and dried in air.
83	Shore material, west side of Andros Island, Bahamas.	0.89	43.47	5.82	40.49	0.98	13.36	85.66	
84	Tongue of the Ocean, Bahamas.	1.05	51.30	1.85	42.31	1.09	4.02	94.89	Depth 822 fathoms, washed and dried over H_2SO_4.
85	Tongue of the Ocean, Bahamas.	1.34	51.05	1.30	41.52	1.40	2.89	95.71	Depth 820 fathoms, washed and dried over H_2SO_4.
91	Mud flat, north side Loggerhead Key, east of Sugar Loaf Key, Florida.	1.04	47.86[3]	1.22	38.93	1.18	2.87	95.95	As received.
97	Near obstruction buoy at southwest entrance to Fort Jefferson Channel, Tortugas, Florida.	1.11	51.02	1.77	42.01	1.16	3.86	94.98	Sample washed and dried in air and over H_2SO_4.

[1] From Vaughan (1918 a, p. 270).
[2] Loss up to 150°, 0.44 per cent moisture.
[3] Loss up to 150°, 6.77 per cent moisture.

Magnesium carbonate averages 1.73 per cent for the entire twenty samples. The maximum amount is 3.07 per cent in sample 145 and the minimum is 0.44 in sample 199.

If $CaCO_3$ and $MgCO_3$ are combined, then 75 per cent of the samples contain over 90 per cent $CaCO_3+MgCO_3$.

It is noteworthy that sample 117, having the highest percentage of calcium carbonate, and sample 199, containing the lowest percentage of either calcium or magnesium carbonate, also show the highest Ca/Mg ratios. In

other words, these two samples are extremes and deviate accordingly from the average ratio value.

The variability shown in table 8 indicates that the average values must not be taken too seriously. However, they are sufficiently accurate to show the general trend.

The average Ca/Mg ratio for all samples is about 76, or expressed as $\frac{CaCO_3}{MgCO_3}$ the value is about 55. The highest ratio, shown by sample number 117 from just outside Marquesas Lagoon, shows that there is about 138 times as much calcium as magnesium. Too much emphasis should not be placed on a single determination, because sample number 63 from inside the same lagoon has a Ca/Mg ratio of 64, which is less than the average. However, when the region is divided into six geographical divisions, there is a semblance of grouping. This information, presented in table 8, shows the lowest Ca/Mg ratio to prevail on the east side of Andros Island. Following in order of increasing ratio are the east side of Andros Island, Tortugas, the west side of Andros Island, South Bight, Florida reef west of Pine Islands, and lastly, Florida reef north of Barnes Sound.

The Corallinaceæ predominate in importance over *Halimeda*, both on the east side of Andros Island and at Tortugas. This explains why more magnesium is being contributed to the sediments of these two localities.

TABLE 10—*Chemical analysis of composite of samples 190 to 193 from behind reef off Cocoanut Point, Andros Island (by Alfred A. Chambers).*[1]

Analysis		Reduced	
Constituent	Per cent	Constituent	Per cent
Loss on ignition....	44.84	SiO_2.....	0.09
SiO_2.....	0.09	$(Al, Fe)_2O_3$.....	0.08
$Fe_2O_3Al_2O_3$.....	0.08	$MgCO_3$.....	5.24
CaO.....	51.56	$CaCO_3$.....	94.59
MgO.....	2.43	$Ca_3P_2O_8$.....	.00
P_2O_5.....	None	$CaSO_3$.....	Trace
SO_3.....	Trace		
Summation...	99.00	Summation...	100.00

[1]From Vaughan (1918 a, p. 263).

ARAGONITE NEEDLES AND CALCIUM CARBONATE EQUILIBRIUM

ARAGONITE NEEDLES

The presence of countless minute particles of aragonite in practically every sample of calcareous sediment taken in these regions has been known for many years. Vaughan first noticed them in 1912 and published photomicrographs in 1917 (Vaughan, 1917, pl. 47, fig. 6). H. E. Merwin identi-

fied the mineral as aragonite. Many of the smaller needles show vigorous Brownian movement. On their presence rests much of the evidence that chemical precipitation of calcium carbonate takes place. Their occurrence is recorded from the shallow mud flats on the western, the lee-side of Andros Island, flats behind some of the Florida Keys, and within Marquesas and Tortugas lagoons. No recognized aragonite needles were recorded from Murray Island, Australia, by Goldman, or from Pago Pago, Samoa, by Bramlette. At Cocoanut Point a few objects believed to be aragonite needles seem to occur, but it is only in the western part of South Bight and off its western end and behind some of the Florida keys where the material occurs abundantly enough to suggest precipitation.

Artificial needles of aragonite, identified by Revelle (1932), were obtained by Gee (1932) by passing air free of CO_2 through sea-water collected at Tortugas. These needles are similar to those figured by Vaughan and have approximately the same dimensions (average about 3μ by 0.5μ). Additional precipitation of aragonite needles and of small spherulites was later produced on a number of occasions by Revelle and Fleming in the chemical laboratory of the Scripps Institution by passing air free of CO_2 through sea-water collected at the pier of the Scripps Institution.

Measurements made by the writer of Revelle's X-ray spectrograms of aragonite needles from sample 177 (west South Bight) and of artificial needles precipitated by Revelle and Fleming were the same for both and for spectrograms of crystallographically determined specimens of aragonite from the mineralogic collection in Berkeley.

Although Vaughan, as early as 1912, had suggested that aragonite needles were deposited in sea-water, Field (1931) thought that they were formed in fresh water on the land and washed from there out over the Bahama Bank. As mentioned before, the precipitation of aragonite needles artificially from sea-water has been accomplished by several people. That needles might be precipitated from fresh water is also a possibility, but the weight of evidence seems to be in favor of a marine origin for the great number of needles observed in these sediments.

BACTERIAL PRECIPITATION OF CALCIUM CARBONATE

In the first part of the paper it was suggested that first attempts to explain the precipitation of calcium carbonate centered about some organic influence such as photosynthetic activity of plants and, more particularly, the production of ammonia by bacteria.

Drew (1914) performed a series of experiments and made bacterial counts which led him to the conclusion that calcium carbonate could be precipitated through the action of ammonia produced by denitrifying bacteria which occurred abundantly at places within the area.

Kellerman and Smith (1914) extended the work of Drew after the latter's unfortunate death in 1913. These two workers devised culture media which

more nearly simulated conditions in the sea than did those used by Drew. The specific organism was isolated and assigned to the genus *Pseudomonas*. Abundant precipitation of calcium carbonate resulted when this organism was grown in association with organisms which produced carbon dioxide.

Lipman (1922), however, concluded from his studies made in Samoa and later at Tortugas, Florida, that denitrifying bacteria do not cause precipitation of calcium carbonate in sea-water.

A more exhaustive study by N. R. Smith in 1922 confirmed the findings made in 1914 and showed definitely that pure cultures from a precipitated bottom mud will in turn precipitate $CaCO_3$ from controlled media. It was indicated furthermore that strongly ammonifying *Vibrios* are probably more important agents than the denitrifying *Pseudomonas*.

Although not seen in dried bottom deposits it is evident, from the work of G. H. Drew, that bacteria occur in enormous numbers in some places and there is no reason to doubt that they are found in the surface layers of the shallow waters of the region as well as in the shallow-water muds. From the surface muds collected in the west entrance to South Bight, Andros Island, Drew found as many as 160,000,000 bacteria per cubic centimeter. This represented one species, *Pseudomonas calcis* (Drew). In describing the mud he says: "The surface layer of the mud for a depth of about six inches is of a creamy white color, but below that it is of a grayish tinge and has a slight odor of sulphureted hydrogen." The implication appears to be that reduction processes are less, or are at equilibrium with oxidation, in the first six inches of mud, but that they predominate below that depth.

Bacteria have been reported in large numbers from Tortugas, Marquesas and various localities by Kellerman and Smith and others.

Bavendamm (1931) found, contrary to Drew's results, that relatively few bacteria occur in the white calcium-carbonate muds off the Andros Banks and off the west coast of Andros, but that on the coast of Williams Island and especially in the Mangrove swamps found inland, the number of bacteria on the surface layers may exceed 16,000,000 per gram, and at a depth of four feet over 2,000,000 per gram. Bavendamm believes "that the bacteriological process of calcium carbonate precipitation may be confined to certain localities" and it is assumed "that mangrove swamps or similar places represent the natural localities for the microbiological calcium carbonate precipitation."

The precipitation of calcium carbonate under artificial conditions by bacteria has been established recently by Williams and McCoy (1934). As was mentioned before, Lipman (1922, 1924, 1929, 1931) doubted that marine bacteria could cause wholesale precipitation of calcium carbonate. The present consensus seems to be that bacteria are not more than accessory factors.

DISCUSSION OF PHYSICO-CHEMICAL PRECIPITATION

In preparing the following statements, information was freely drawn from a paper by R. R. Revelle and R. H. Fleming (1934).

In the Mass law expression, $[Ca^{++}] \cdot [CO_3^{--}] = K'_{CaCO_3}$. The constant, K'_{CaCO_3} is the apparent or stoichiometric solubility product constant of calcium carbonate in sea-water for any given salinity and temperature in equilibrium with solid calcium carbonate.

This constant is altered by the presence of strong electrolytes and the concept of ionic activity has been applied by recent workers for calculation of the activity coefficient of calcium ion in sea-water.

In fixing values for K'_{CaCO_3} it is necessary to determine the quantities of calcium and carbonate ions which may be present in equilibrium with solid calcium carbonate under any particular set of conditions. Calcium may be determined directly but carbonate is deduced from CO_2 relationships by means of equations derived from the mass law equation.

When carbon dioxide is added to or extracted from sea-water, the following elementary equations represent the change:

$$(1)\quad CO_2 + H_2O \leftrightharpoons H_2CO_3$$
$$(2)\quad H_2CO_3 \leftrightharpoons H^+ + HCO_3^-$$
$$(3)\quad HCO_3^- \leftrightharpoons H^+ + CO_3^{--}$$

Equations No. 2 and No. 3 may be expressed in the following form:

$$\frac{[H^+] \cdot [HCO_3^-]}{[H_2CO_3]} = K'_1 \text{ and } \frac{[H^+] \cdot [CO_3^{--}]}{[HCO_3^-]} = K'_2$$

If the quantities in brackets represent molar concentration, then K'_1 and K'_2 are the proportionality constants of the first and second ionization steps, respectively, of carbonic acid.

The expression: $pK'_2 = pH - \log \frac{B_{CO_2} - CO_2}{2\,CO_2 - B_{CO_2}}$ gives the approximate relationship between K'_2 in sea-water, total carbon dioxide, hydrogen ion concentration, and that part of titratable base which is balanced by $_2CO_3.H$ Titratable base is represented by B_{CO_2}, CO_2 = total carbon dioxide, pH and pK'_2 = the negative logarithms of H^+ and K'_2. The quantities CO_2 and pH are determined analytically, while B_{CO_2} may be deduced from them and from determinations of total titratable base.

The average Ca^{++} and CO_3^{--} ion-product obtained by Revelle and Fleming was 2.37×10^{-6} and, multiplying by 0.015, the approximate value for the activity coefficient of carbonate ion, a value of 3.6×10^{-8} results.

Johnson and Williamson (1916) gave 0.81×10^{-8} as the solubility product for calcite at 30° C. in pure dilute carbonate solutions. The activity coefficient of the calcium ion in sea-water, according to Revelle and Flem-

ing, is in the neighborhood of 0.225. This agrees well with a value of 0.27 obtained by Hastings, Murray and Sendroy (1927) for salt solutions of which the ionic strength was 0.16.

The conclusion reached by Revelle and Fleming was that variations of pH within the normal range of sea-water (7.7-8.2) were more important in the precipitation or solution of calcium carbonate than changes in salinity.

An apparent relationship between the second dissociation constant of carbonic acid in sea-water and pH may be due to the presence of boron compounds. When a correction is applied for them, the calculated values of pK'_2 at any pH are constant within the limits of error.

An important paper by Moberg, Greenberg, Revelle and Allen, entitled "The buffer mechanism of sea-water" has appeared recently (Bull. Scripps Inst. Ocean., tech. series, 3, pp. 231-320, 1934.) Some pertinent facts bearing on the fundamentals of calcium carbonate precipitation have been condensed from it and are given below:

1. Hydrogen ion concentration of sea-water is relatively constant and varies only within rather well-defined limits. The complex physical and chemical factors which determine its concentration are included under the term buffer system or buffer mechanism of sea-water.

2. The chemical components of this mechanism are the salts of weak acids, especially carbonates and bicarbonates and, to a lesser extent, borates and silicates.

3. Three important variables of buffer mechanism are pH, titratable base, and total carbon dioxide content.

4. Theoretical treatment of data requires the use of a concept of ionic activity in calculating the first and second dissociation constants of carbonic acid in sea-water.

5. The factors considered interconnected in buffer mechanism are pH, carbon dioxide tension, total carbon dioxide content, titratable base, bicarbonate ion content, carbonate ion content, dissolved free carbon dioxide, vapor pressure of sea-water, solubility of carbon dioxide in sea-water, hydrostatic pressure, temperature and salinity. In addition, two other factors, "non-volatile" buffers, such as borates and divalent ions, must be included. Many of these factors are relatively constant, therefore, by measurement of the three major variables, namely pH, titratable base and total carbon dioxide, an approximate formulation of the system is possible.

6. Three convenient conditions are recognized in treating the system. (*a*) When appreciable quantities of only bicarbonate and carbonate are present with a negligible amount of free carbon dioxide, the usual condition of sea-water in the surface layer and down to fifty meters or more, depending on locality and other factors; (*b*) when carbonate concentration is negligibly small and only bicarbonate and free carbon dioxide, both as H_2CO_3 and unhydrated are present; and (*c*) the general case when all three substances are present.

7. Generalized mass law equations, applicable to pH value, for quantitative study of the buffer mechanism of sea-water in terms of hydrogen ion concentration, titratable base, and total carbon dioxide have been developed by these workers.

8. The large difference between the solubility product constants of calcium carbonate in sea-water and in pure water is explained as being partly due to the low activity coefficient of the carbonate ion in sea-water.

It is clear that an increase of pH represents removal of CO_2 and corresponding increased concentration of CO_3^{--} ion. Thus pH, if base remains constant, becomes an apparent index to the quantity of CO_3^{--} ion present. Conditions which tend to remove CO_2 from sea-water would be expected to prevail if calcium carbonate is being precipitated. It is now possible, by applying recently developed equations, to test the conditions at Tortugas to discover if precipitation could actually take place. The data obtained by Dole (1914) are believed to be both accurate and trustworthy, consequently they will be used as basic measurements. By converting his values for total carbon dioxide and total alkalinity from grams/kilogram to millimoles/liter one can write out the following table:

	Cl	Salinity	CO_2 (total)	Base (total)
	grams/kilogram		millimoles/liter	
Minimum	19.93	36.00	2.312	2.518
Maximum	19.95	36.04	1.915	2.448
Average (52 determ.)	19.93	36.01	2.009	2.418

An examination of surface water temperatures recorded at Tortugas over a period of thirty years showed that the average temperature for the interval under discussion was about 27° C. The fundamental equation employed is:

$$\frac{[Ca^{++}] \cdot [CO_3^{--}]}{K'_{CaCO_3}} = 1.0 \text{ at saturation.}$$

The use of this equation and methods for obtaining the numerical values for the respective terms is given by Revelle (1934). However, in Revelle's formula for obtaining H^+ there is a typographical error; the corrected equation should read:

$$(aH^+)^2 - K'_1\left(\frac{\Sigma CO_2}{B_{CO_2}} - 1\right)aH^+ - K'_1K'_2\left(2 \times \frac{\Sigma CO_2}{B_{CO_2}} - 1\right) = 0.$$

Temperature is one of the important factors in figuring percentage of saturation for calcium carbonate. Surface temperatures at Fort Jefferson wharf, Tortugas, often reach 33.0° in July and August. Temperatures ranging from 33.0° to 38.0° have been recorded in the tidepools on the reef at Tortugas (Vaughan, 1918b). There is reason to believe that even these extreme temperatures are exceeded at times.

High salinity values are also to be considered in equilibrium conditions. At Fowey Rocks, on the reef tract, the salinity was about 36.00 parts per thousand. Vaughan (1917, p. 936) showed that off the west side of Andros

Island the salinity of a spot sample of sea-water was 38.86 per mille, while that of the Tongue of the Ocean on the east side was about 36.50. The spot sample came from over a submarine flat having a width of 60 nautical miles and a maximum depth of 18 feet. The agitated surface and relative high temperatures, together with the high salinity all favored the precipitation of calcium carbonate. M. A. Black (1933) stated essentially the same thing for this flat.

Equilibrium conditions might be disturbed in this manner: the water, being very shallow, has a high pH, hence a high CO_3^{--} content from top to bottom because of mixing and removal of CO_2 from water by marine plants. This fact, coupled with high temperature and high salinity, brings about conditions of saturation for calcium carbonate.

Whether or not there is solution when conditions of undersaturation prevail is not known, but if such were true it is reasonable to suppose that the smallest particles would dissolve first. The ratio of solution to precipitation might be about equal or slightly in favor of precipitation at Tortugas, but on parts of the Bahama Bank and in South Bight, where higher salinities and higher average temperatures probably prevail, the ratio is perhaps shifted definitely in favor of conditions of precipitation. The computed values are tabulated below:

	$[Ca^{++}]$ $(\times 10^{-3})$	$[CO_3^{--}]$ $(\times 10^{-3})$	K'_{CaCO_3} $(\times 10^{-6})$	$\frac{[Ca^{++}] \cdot [CO_3^{--}]}{K'_{CaCO_3}}$	Per cent saturation
Minimum	11.05	0.2172	2.57	0.933	93.3
Maximum	10.994	0.401	2.57	1.630	163.0
Average	10.971	0.313	2.57	1.335	133.5

These values, which tend to be conservative, show that while occasionally the sea-surface water at Tortugas is slightly under-saturated, most of the time during the summer months there is a high degree of oversaturation. Since aragonite needles in the bottom deposits offer abundant seeding opportunities there should be some precipitation. The presence of the needles themselves indicate that physico-chemical precipitation has already occurred.

The value for K'_{CaCO_3}, corrected for a temperature of 27° C., was obtained from the calculations of Revelle and Fleming (1934). If the figure given by Wattenberg (1933) had been used, the values of $\frac{[Ca^{++}] \cdot [CO_3^{--}]}{K'_{CaCO_3}}$ would, have been several times as large. Since conditions for crystallization from solution are obviously present, it appears that a figure which shows many times over-saturation is incorrect and that Wattenberg's constant, K'_{CaCO_3} is too small.

DREWITE, VAUGHANITE

Field (1919) proposed that the fine-grained deposits in Tortugas lagoon be called "drewite," but since no physical, chemical or other descriptions

TABLE 11—*Hydrographic data*

A. Surface water temperatures in the Bahamas
by Dr. Paul Bartsch, U. S. National Museum.

Date	Hour	Air temperature	Water temperature
1912			
	h. m.	°F.	°F.
May 13	5 30 a. m.	75.0	....
	10 00 a. m.	....	83.0
14	5 30 a. m.	77.0	....
	6 30 a. m.	....	80.0
	9 00 p. m.	80.0	80.0
15	6 00 a. m.	?	79.0
	12 20 p. m.	83.0	84.0
	9 00 p. m.	81.0	80.0
16	6 30 a. m.	80.0	80.0
	9 00 p. m.	80.0	80.0
17	7 00 a. m.	75.0	80.0
	9 00 p. m.	80.0	80.0
18	9 00 p. m.	80.0	80.0
19	6 30 a. m.	80.0	80.0
	9 00 p. m.	80.0	80.0
20	6 30 a. m.	80.0	80.0
	9 00 p. m.	80.0	80.0
21	6 30 a. m.	80.0	80.0
	9 00 p. m.	80.0	80.0
22	6 30 a. m.	79.0	80.0
	9 00 p. m.	79.0	80.0
23	6 30 a. m.	79.0	80.0
	9 00 p. m.	81.0	81.0
24	9 00 p. m.	80.0	81.0

Subsurface temperatures, Bahamas, Drew (1914, pp. 34, 35.)

B. Drew's Station 1. 6 knots east of Golding Cay. Latitude 24° 14′ N, Long. 77° 28′ W. 822 fathoms. May 9, 1912 (Vaughan, b. s. 84)

Depth in fathoms	Temperature
0	26.9°
10	25.9°
50	25.14°
100	22.0°
200	17.13°
400	8.98°
600	4.70°
822	3.97°

C. Drew's Station 2. 14 knots east of Golding Cay. Lat. 24° 12′ 0″ N, Long. 77° 21′ 30″ W. (Vaughan, b. s. 85)

Depth in fathoms	Temperature
0	26.3°
10	26.4°
50	24.89°
100	22.63°
200	17.42°
300	14.32°
400	9.79°
820 (bottom)	4.15°

were given, its usage has been subject to criticism. Likewise, Kindle (1923) suggested the term "vaughanite" for fine-textured limestones which are supposed to represent indurated "drewite." He further used the term "drewite" for typical shallow-sea deposits of the Great Bahama Bank west

of Andros Island. Vaughan (1924, p. 316) pointed out the "drewite" of Field is not the "drewite" described by Kindle, and showed that the deposits from Tortugas lagoon are coarser grained and contain fewer aragonite needles than those from off South Bight, and that the two deposits are quite dissimilar in other ways. The origin of Tortugas fine-grained mud is thought to be for the most part minutely comminuted fragments of organic tests, with possibly some precipitated material, while the material from off South Bight, *i.e.* Kindle's "drewite," appears to be largely calcium carbonate precipitated from sea-water.

MINERALS

QUANTITY AND DISTRIBUTION OF QUARTZ

Sparsity of mineral grains other than those composed of calcium carbonate is one of the first observations which one makes on studying these deposits. This relative absence is particularly true in the Bahamas and from Marquesas westward. That portion of the Florida reef tract east of Marquesas, on the contrary, has considerable quartz. Sample 198, collected just off Cape Florida near Miami, contained 47.2 per cent minerals, of which quartz constituted practically the entire amount. Of the samples described by Matson and used here for comparison, a similar amount is recorded around Miami. As one follows the reef tract southward and westward the amount of quartz decreases rapidly and almost proportionately to the distance. Even a few hardy grains reach Tortugas, as is seen in table 13 b, c, d.

It is possible that a little of the quartz may be derived from underlying formation, as may also be the case of some oolite grains at Marquesas, the Quicksands and Tortugas. Matson (See Vaughan, 1910, pp. 126-129) has outlined the known possible sources of siliceous material in Florida. In northern Biscayne Bay the quartz appears to be derived from similar material overlying or contained in the Miami oolite in adjacent regions. Wells sunk as far south as Key Vaca reveal an abundance of siliceous sand believed to be Miocene and Pliocene.

Obviously the southwestern reef tract area was receiving siliceous deposits at that time from continental land masses to the north. The Pleistocene limestones of the mainland rest on an arenaceous foundation.

Although the quartz now present in the bottom deposits is believed to be derived from nearby sources, Martins (1931a) locates the primary source "in the Piedmont and Appalachian Mountain region where sand has been and is being formed by the decay of hard rocks . . . sand which has been carried down to the sea by the . . . rivers of Georgia and the Carolinas is gradually shifted to the southward by shore currents and wave action along the Atlantic Coast. Thus there is a constant replenishing supply of sand on the east coast of Florida."

Again, the same writer (1931b) states that feldspar grains found in the vicinity of Miami have apparently been transported at least 400 miles along the shore, plus another 200 miles by river, while some of them may have come much greater distances.

MacCarthy (1933) has examined some beach sands from near Miami and, in discussing Martins' figures, comments that the angularity of quartz grains is greater than that of sands from the Delaware-Chesapeake Bay area. Some conclusions reached by MacCarthy are given below:

"1. No evidence of any rounding of the quartz grains composing the beach sands can be detected during a straight-line movement of about 130 miles along the coast and only a comparatively slight degree of rounding can be shown for grains which are known to have migrated several hundred miles.

"3. The coarser grains tend to be more rounded, although a fair amount of rounding may be present even in the 0.177-0.149 mm. size.

"4. The most prevalent grain sizes also tend to be the more rounded.

"5. This peculiar distribution of roundness seems to be caused by a sorting action of the waves and currents which move the sand. . .

"7. Sedentary sands appear to be both more angular and more variable in respect to roundness of individual grains than are beach sands.

"8. Assuming that the sedentary sand studied is typical of the primary material from which the beach sands were derived, the Florida sands show much less modification of their primary characteristics than do the Delaware-Chesapeake Bay sands, and hence must be (in the sense of an evolutionary development) much younger than they.

". . . (It is implied) that such transportation must come about through suspension in the surf rather than by traction along the bottom."

The writer can not agree entirely with either Martins or MacCarthy that these beach sands have been transported along the present coast by waves and currents. Quartz grains observed at Tortugas and other localities along the reef tract are usually as angular as, or sometimes more than, those examined from the northern part of Biscayne Bay. Occasionally, quartz grains stained interstitially with iron oxide are seen within the lagoons. It appears more probable that, since angular quartz grains are common in the oolite which forms most of the coastal country rock, they are derived from it. Ferric oxide is not abundant in the rocks, but there is enough to produce stains.

OTHER MINERALS

Vaughan (1918, p. 288) stated that no appreciable terrigenous material (SiO_2 and Al_2O_3) reached the Bahamas. This is substantiated by the present study. A few angular grains of supposedly terrigenous origin are seen occasionally and a small amount, widely distributed and thought to be wind blown, occurs in grades which range downward in size from 0.05 to 0 mm. The olivine is likewise believed to be wind derived. Magnetite and mica are both scarce, particularly the latter. The probability that

they are wind blown must be considered, partly because winds of sufficient velocity to carry such particles from Cuba, Haiti and other nearby sources occur annually, and partly because these two minerals are somewhat unstable and less likely to withstand prolonged transportation by waves and currents. Their relative angularity gives further support to the suggested eolian transport.

Gypsum occurs well distributed and in sharply angular grains. Its presence is probably due to precipitation from sea-water. This mineral precipitates rather easily, as is shown by its occurrence throughout many places in the world, both in recent and geologically ancient deposits.

An interesting occurrence of green epidote in samples from the northern part of Biscayne Bay is recorded. This mineral, as well as garnet, appears to have persisted through an erosional cycle along with the quartz grains. However, Lapparent (1924) has recorded epidote as an authigenic species in Devonian Radiolarian rocks from the Valley of the Bruche.

Glauconite and chert are both present locally, but since they are not unknown in the country rock they are assumed to be derived terrigenously. It is not known how many cycles of erosion glauconite will withstand, but the very few grains in these deposits appeared to be old, eroded, and definitely not of authigenic origin.

PYROCLASTICS AND ORGANIC CARBON

Volcanic glass is encountered frequently in silt and clay fractions (0.05-0 mm.). Some ash fragments are rarely seen in the sand grades. Sharp outlines and jagged edges attest to the freshness of this material. Identification by inspection was easy and confirmation was obtained by measuring the index of refraction and comparing with indices of known volcanic glasses (George, 1924). All of the volcanic material present was classed as wind borne.

Pieces of coal were found in abundance in bottom material collected from Tortugas lagoon. This was not surprising when it is known that a coal shed was located on Garden Key at Fort Jefferson. Currents have strewn the coal all over the lagoon bottom and even outside of it. The presence of this coal made it necessary to test all grains with a magnetized needle in order to distinguish between it and magnetite. Ashes, also thought to be of local origin, were found frequently in deposits which were collected from near human habitations.

Even more widely distributed were organic grains blackened either by fires or by soot resulting from fires. The coatings thus acquired could be removed by further heating of the grains in the flame of a burner. Microchemical tests for manganese gave negative results, but the reagents used may not have been sensitive enough to show reaction to such small quantities.

COSMIC SPHERULE

A single sideritic spherule was taken from sample 206a from off Key West, Florida. This spherule showed some oxidation on part of its surface, the ferrous carbonate being converted in part to ferric hydroxide in the form of limonite. The principal significance here, aside from the rarity of such cosmic particles, is that oxidation is going on at this place in very shallow water on the surface of the bottom.

FOSSILS AND MINERALS DERIVED FROM OLDER FORMATIONS

KINDS AND RELATIVE ABUNDANCE

Mention has already been made of supposed land-derived minerals such as quartz, epidote, garnet, chert, olivine and glauconite, coal, ash, carbonaceous grains and limonite. With few exceptions every sample examined had many old eroded grains of organic and inorganic origin mixed with fresh comparatively uneroded material. Specifically, there was little or no difference detected in such organisms as foraminifera, mollusks, or any other for that matter, except that fresh specimens predominated in numbers over the eroded ones. Examination of land material showed the origin of the older darker tests. Their preservation in older rocks appears to weaken the structure of tests so that even though they withstand transportation into the second cycle, there is considerably more breakage than in fresh forms. Only when fresh organic tests are burned or otherwise altered do they become indistinguishable from the fossils.

SECONDARILY DERIVED OOLITES

Oolites derived from Pleistocene formations and washed into recent deposits are sometimes so fresh that one wonders if they might not be authigenetically formed. The country rock, in place, is equally fresh appearing. Most oolites are, however, somewhat eroded or blackened by the time they are incorporated into recent bottom deposits.

DISCUSSIONS OF MECHANICAL ANALYSES

SCOPE OF MECHANICAL ANALYSES

The mechanical condition of a sediment at any given time is the resultant of two groups of agencies operating against each other, namely, agencies of disintegration and reduction of size, and those of aggregation. Of the first type, one would identify such inorganic agencies as waves, currents, solution, abrasion and other mechanical effects due to general friction caused by movement of particles against each other. Organic agencies falling into this classification include boring organisms such as algæ, sponges, mollusks, worms and many other things. Sand feeders reduce particle size by grinding and also by chemical action of their digestive

fluids. The second type of agencies causes aggregation and increase of particle sizes. Growth of organisms and chemical accretion and cementation of smaller units are readily called to mind as typical examples of this group.

Sorting of grain sizes is determined largely by intensity of mechanical agencies and by growth or predominance of individual species of organisms. Organic particles observed in any given grade usually occur as (1) the original form, such as entire tests, spicules, spines, or plates; (2) particles which have a secondary form as a result of disintegration of the original body, or secondary aggregation of many organic fragments, such as cemented aggregates and certain worm tubes.

The sediments described in this paper have, for the most part, not been subjected to prolonged transportation, but local mechanical erosion has been and is of considerable intensity. Except in the reef tract north of Key Largo, there is general absence of hard gritty material such as quartz.

Corrasion, which normally produces large quantities of silt and clay particles, is to some extent replaced in this region by physico-chemical precipitation, thus supplying the same sizes in a different manner. The supply of larger-sized particles is furnished principally by the massive skeletons of calcareous organic structures which are found in profusion. These structures are found in these sediments in the place usually occupied by transported rocks and minerals derived from land masses in other regions, but low relief and absence of large rivers make the latter source of supply almost negligible for both the Florida mainland and the Bahamas.

As mentioned before under the caption of "Method of Procedure," mechanical analyses were made in the laboratories of the Bureau of Soils, United States Department of Agriculture. The methods used are described by Fletcher and Bryan (1912) and additional comments on the accuracy of such and related methods are well discussed by Wentworth (1926, 1927) and Galliher (1933).

Table 12A and B gives for all samples considered the percentage by weight of all particles included within certain definite size limits indicated at the top of the columns. These same weight percentages are plotted as histograms and are presented in plates which follow.

DISTRIBUTION OF PARTICLE SIZES IN THE VARIOUS REGIONS

South Bight (fig. 6A)—In and around the west entrance to this shallow waterway occur sediments which differ from all others examined, inasmuch as silt and clay particles usually make up a large part of the total weight. Samples 79, 83, 87, 88, 175 to 183 are all from this area, and all except 176a and 182 show a maximum in the silt and clay. No. 176a is shore material and 182 was collected on the north side of Steamboat Cay. Both contain quantities of molluscan, algal and foraminiferal remains in addition to a relatively large percentage of oolites. These two samples differ from the other twelve principally because of a removal of fine particles.

That portion of the shallow flat which lies immediately off the west entrance is uninfluenced by strong currents, hence fine particles are not washed away.

Fig. 6—A. Histograms of samples from west South Bight, Andros Island. B. Histograms of samples from east South Bight (Golding Cay entrance), Andros Island. (For scale see fig. 7.)

Farther out on the Bank, agitation of water stirs up the sediments and brings into suspension the fine particles, which are subsequently carried off by currents into deeper water.

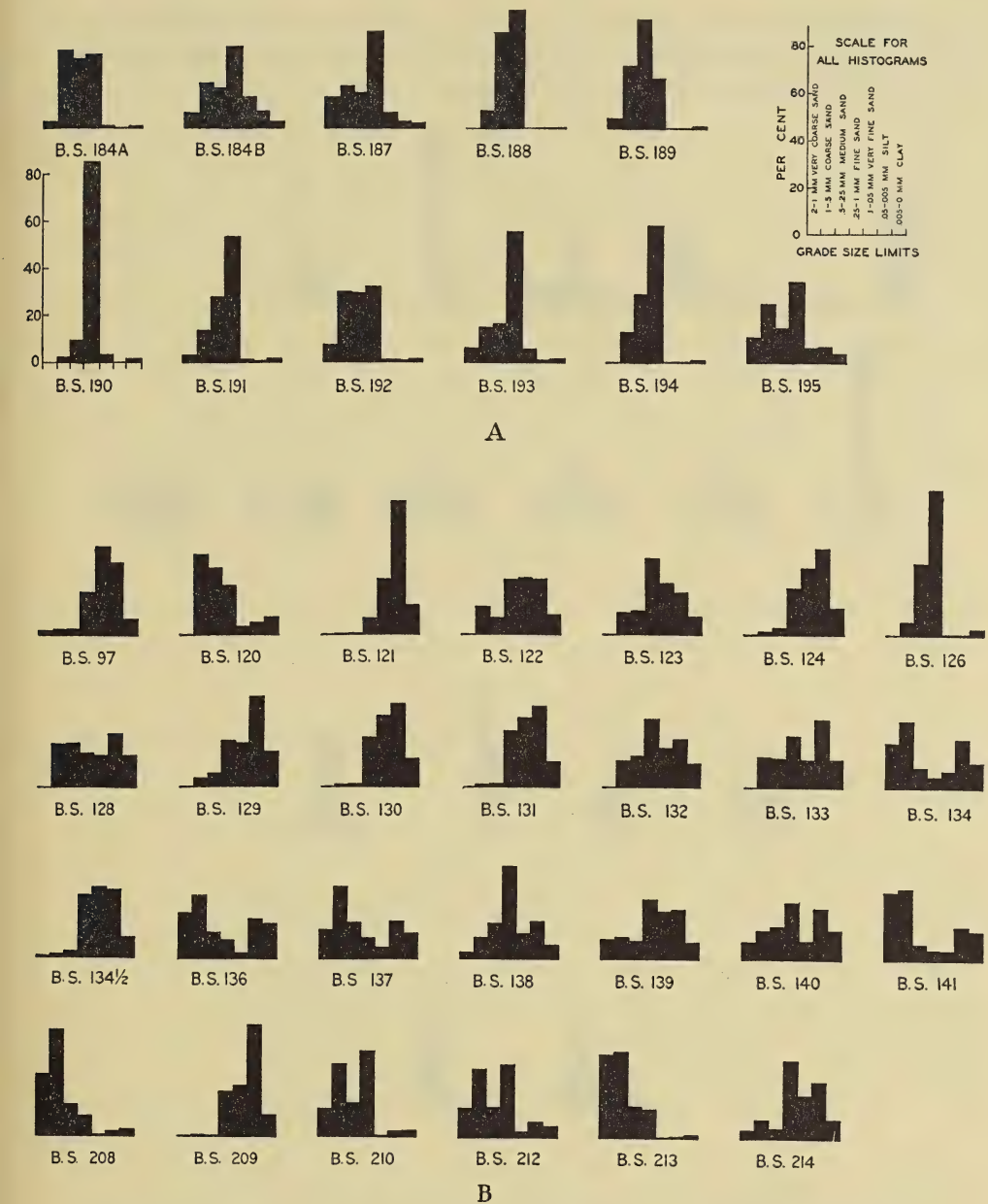


FIG. 7—A. Histograms of samples from behind the reef at Cocoanut Point, Andros Island. B. Histograms of samples from Tortugas and vicinity, Florida.

Golding Cay entrance (fig. 6B)—This eastern entrance to South Bight, represented by 23 samples, is a tide-swept area where most of the fine particles are carried away, leaving poorly sorted sands on the south bank and well-sorted material in the center and in the channels. A high degree

of sorting is shown by sample 154; the less perfect sorting shown by 149, 151 and 152 is rather characteristic of deposits whose sand grades are determined by predominating organic species.

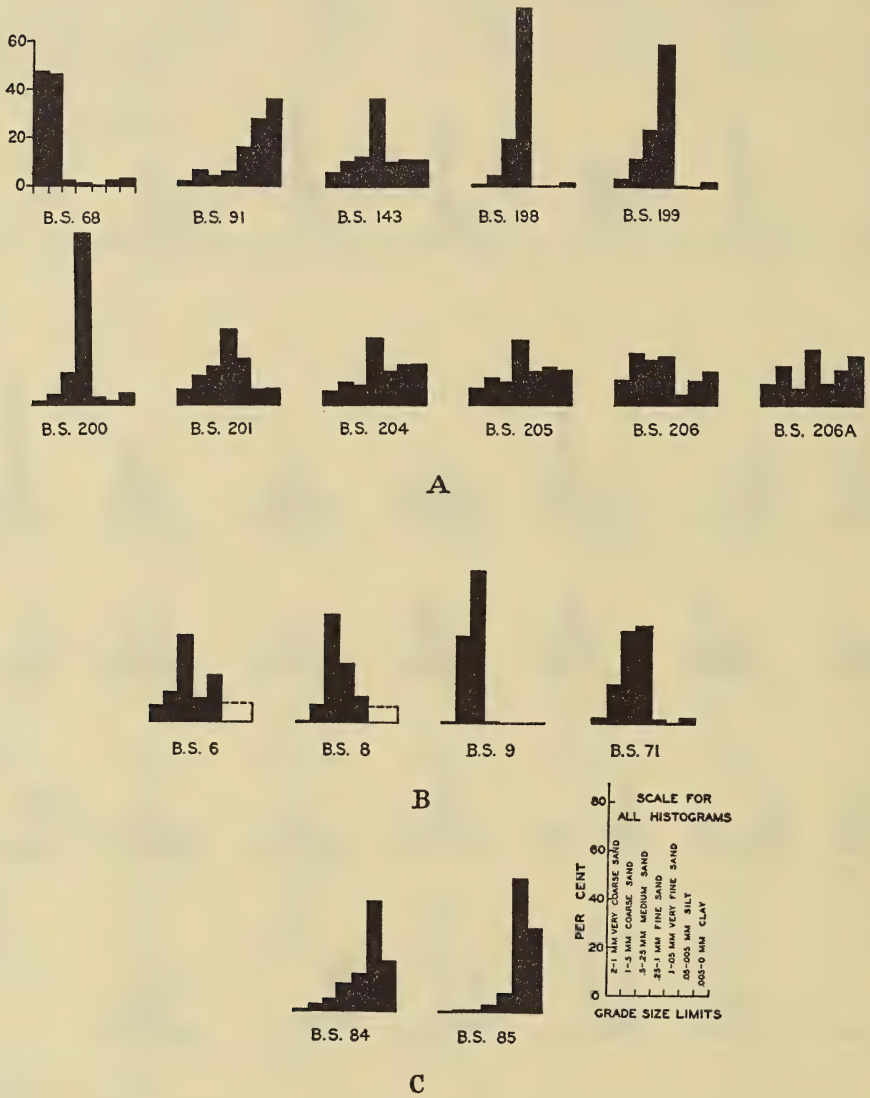


Fig. 8—A. Histograms of samples from Florida reef tract, east and north of Key West. B. Histograms of samples from Great Bahama Bank. C. Histograms of samples from Tongue of the Ocean.

Behind the reef at Cocoanut Point—Goldman (1926) has already described in considerable detail samples 190 to 193 from this locality. The mechanical analyses of his samples, together with seven others, are shown histogrammatically in figure 7A. There is considerable similarity between

these and samples collected in Golding Cay entrance. Both localities are on the same side of Andros Island, the same wind directions prevail across the reef in both places, and except for more active currents in Golding Cay there is little distinction to be made between them.

Great Bahama Bank (fig. 8B)—The four samples plotted show one poorly sorted sand, No. 6; one fairly well-sorted sand, No. 8; and two well-sorted specimens, Nos. 9 and 71. The source material for No. 71 is oolitic limestone, hence the sorting is to some extent determined by the source, but agitation of water over the Great Bank insures that few fine particles will be deposited.

Tongue of the Ocean—Only two samples were available for examination. Their histograms are represented on figure 8C. Both 84 and 85 are Globigerina Oozes. Foraminiferal tests, because of their size, contribute a relatively larger proportion of coarser particles to sample 85 than to 84. Their histograms are somewhat similar to those of the deposits off the west end of South Bight.

Florida reef tract north and east of Key West (fig. 8A, 9)—The variability of the currents among the keys is reflected in the different types of histograms. Well-sorted sediments, such as are represented by samples 10, 11, 198 and 200, are similar to Golding Cay sample 154, Cocoanut Point 190, and 59 from the Quicksands. Removal of finer particles by currents, tides and waves is shown in most cases. Quartz, instead of calcium carbonate, usually predominates in the silt and clay fractions in those sediments of the reef tract north of Key Largo which contain any appreciable amounts of finer material.

Marquesas lagoon (fig. 10A)—These samples are indicative of considerable silting and quiescence on a floor of uniform depth in a protected area. Prominent maxima are shown in samples 62, 63, 115 and 117. Silt and clay are more or less protected from removal by sorting agencies. The quantity of fine material may indicate that some of the mud-choked troughs are filled in part with precipitated calcium carbonate. Samples 110 and 115 represent, respectively, a depth of 9 feet and the surface of a core sample taken at the same spot near the edge of Marquesas lagoon. The former shows that at least locally more silt and clay were being deposited in the past than is the case today.

The Quicksands (fig. 10B)—Strong wave action is indicated here by removal of most of the silt and clay particles from the sediments, with good sorting of the sand grades. Sample 60, being somewhat nearer the protecting ring of Marquesas lagoon, has had less of its finer fractions removed.

Tortugas (fig. 7B)—At Tortugas the finer particles (silt and clay) are found in the almost enclosed marginal pockets, such as Bird Key Harbor, and in the troughs of the lagoon. Sample 121, collected in Bird Key Harbor, contains the largest amount of silt and clay of any sample examined from this locality. Sample 124 from Fort Jefferson Channel is similar.

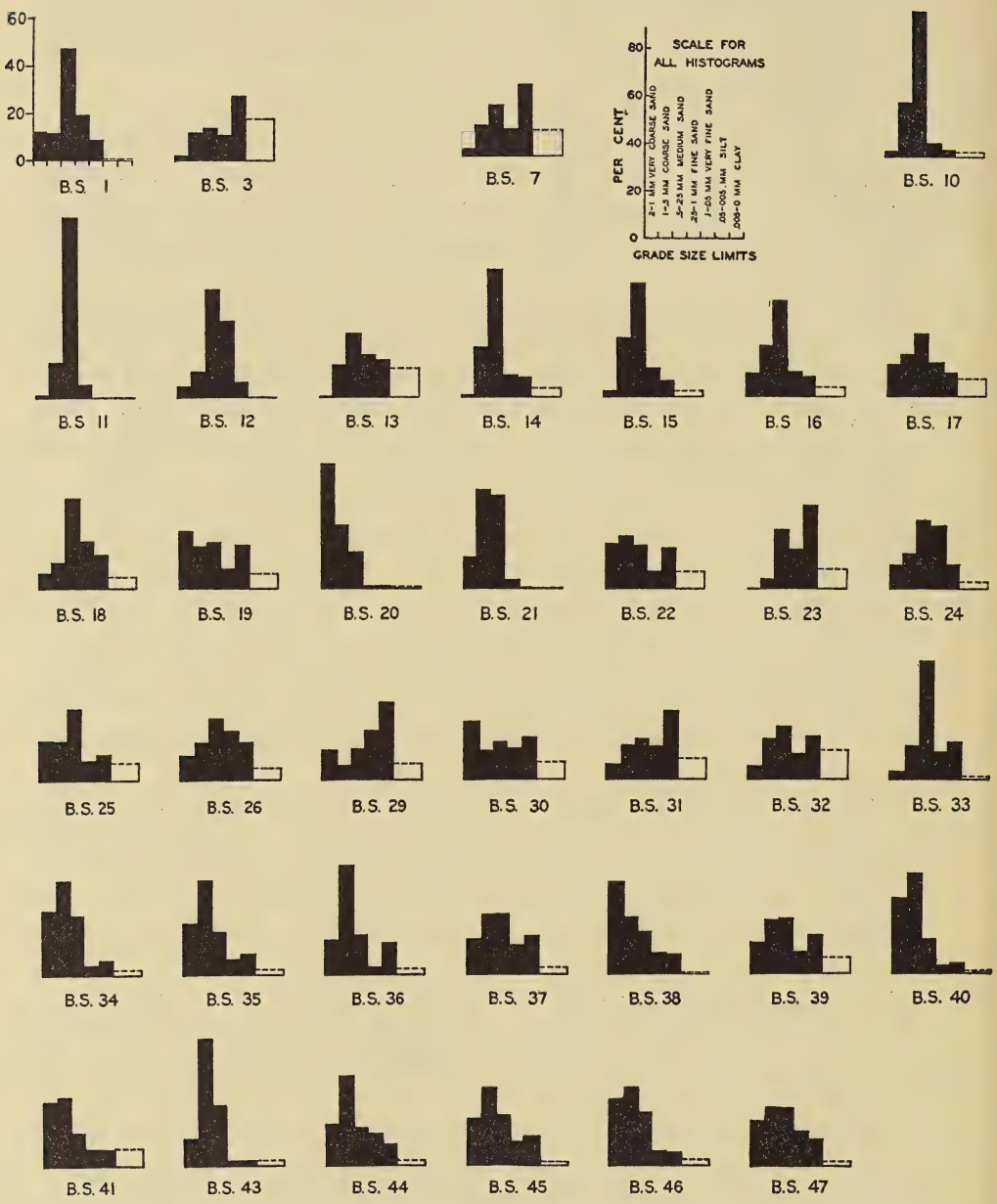
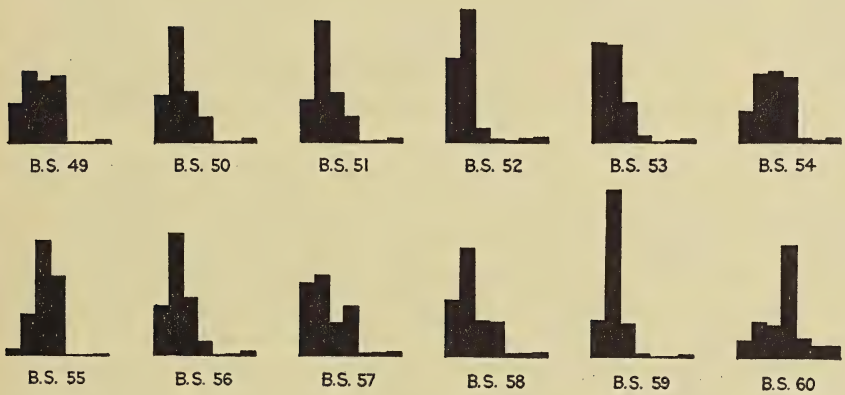


FIG. 9—Histograms of samples from Florida reef tract, east and north of Key West.

Numbers 126, 137 to 141, which were collected on slopes, contain almost no silt and clay, but 97, 129-131, 133 and 134½ are all in troughs and are composed chiefly of fine particles. Waves and currents remove the fine particles from sediments on the slopes and deposit them in the troughs.

This distribution according to size agrees with the relations of texture to bottom configuration which Trask found to apply to the distribution of the sediments in the Channel Islands (Trask, 1932, pp. 88-95). The condition of sediments on the slopes is in direct contrast to that of the sediments near shore on the lee side of Andros Island off the west end of South Bight, where the fine particles are not removed by current action.

A



B

FIG. 10—A. Histograms of samples from Marquesas, Florida reef tract. B. Histograms of samples from the Quicksands, Florida reef tract.

DESCRIPTIONS OF SOME OTHER RECENT MARINE CALCIUM CARBONATE SEDIMENTS

Murray Island, Australia—The Great Barrier Reef of Australia is an area of wide-spread calcareous deposition. Vaughan and Goldman (1918a) published detailed descriptions of six samples, five of which represented a section across a reef and one represented sand cast upon a reef 1700 feet from shore, all being from Murray Island, Australia. All of the usual

groups of organisms have contributed material to the deposits except that there are no Alcyonarian spicules. Little or no chemically precipitated calcium carbonate was detected. In order of importance, the constituents were: (1) coralline algæ; (2) foraminifera; (3) madreporarian corals; (4) mollusca. Essential differences between Murray Island samples and those from Florida and the Bahamas are, first, no aragonite needles are found in the former region, and, second, alcyonarian spicules are common in the latter region.

Pago Pago Harbor, Samoa—Bramlette's (1926) description of conditions in Pago Pago Harbor show that although calcium carbonate sediments are forming, they are not chemical precipitates, but are rather due to accumulation of tests of both reef-forming and non-reef-forming organisms mixed with detritus brought down by streams which flow into the harbor. Here, as elsewhere, calcareous algal reefs predominate in volume over coral reef structure. The genera which represent the plant and animal remains are rather similar for both Pago Pago Harbor and most parts of the Florida-Bahamian regions.

Funafuti Atoll—Cuttings from the well-known boring put down by the Royal Society to a depth of 1114 feet have been studied very carefully by a host of workers, notably Judd, Cullis, David and Sweet. Organic remains were encountered throughout the entire column. The magnesium relationships have yielded considerable information which may be of application to the dolomite problem. Coralline algæ, found abundantly throughout the section, are suggested as being the source of magnesia, since the genera *Lithothamnium* and *Lithophyllum* are known to contain considerable quantities of it.

The column of rock at Funafuti, based on magnesium content, is divided by Clarke and Wheeler (1922, p. 59) into three zones. Zone No. 1, which includes the first 25 feet, contains organisms similar to forms now living off shore. Concentration by leaching is seen. From 25 feet down to 640 feet the rock is limestone with little magnesia. The lowest zone, 640 feet to the bottom, represents dolomite, and the dividing line between it and the limestone above is sharply defined. In 3 feet the percentage of magnesium carbonate rises from 2.44 to 26.33. Comparison of surface forms with those from the Floridian and Bahamian regions shows a similarity. Concentration of magnesia is not definitely shown at Cocoanut Point but neither can it be demonstrated at the surface for Funafuti. Predominance of calcareous algæ in recent deposits should cause one to reflect on the possibility that future deposits of dolomitic limestones, such as occur beneath Funafuti, may be the result.

Maiao Island, South Pacific—Perhaps the most similar calcareous bottom sediments examined from outside the region under discussion occur on a raised coral reef and within an inclosed lagoon at Maiao, in the South Pacific. Two samples of Maiao sediments were described by the author

for Professor Howel Williams (1933, pp. 78-80). The percentage of calcium carbonate in the two samples was 97.83 and 98.01, respectively. The most interesting feature was that aragonite needles were present, thus indicating precipitation. Organic remains constituted about 70 and 65 per cent, respectively, of which calcareous algæ amounted to 32 per cent in one case and 28 per cent in the other.

Calcareous beach on the coast of Maine and at John O'Groats, Scotland—Recent marine calcareous beaches may occur in high latitudes. Two examples are described, one on the coast of Maine by Raymond and Stetson (1932), and the other at John O'Groats, Scotland, by Raymond and Hutchins (1932). 60 to 70 per cent of broken calcareous shells, chiefly of *Mytilus*, *Balanus*, and spines of *Strongylocentrotus*, accumulated on the Maine beach because of favorable hydrographic conditions, limited wave action and small influx of inorganic material. The Scottish beach is somewhat similar in appearance to the one on the Maine coast. Both beaches are present only because of exceptional conditions which prevail in a restricted locality.

SUMMARY

A. Determinations of the quantities of material derived from organic and inorganic sources have yielded the following results:

(1) Coralline algæ, collectively, are shown to be the organic group that makes the largest contribution of organically secreted calcium carbonate.

(2) Next in order of magnitude are the mollusks, followed in descending order by foraminifera, madreporarian corals, alcyonarian spicules, worm tubes, crustacean fragments, and Bryozoa.

(3) The principal non-calcareous mineral is quartz.

(4) Quantitative counts of alcyonarian spicules show that they are relatively minor components of the sediments, being exceeded by madreporarian fragments in a ratio of about 2.5 to 1.

(5) Terrigenous minerals are remarkably scarce, except on the northern part of the reef tract where quartz is abundant. Their source is believed to be from the mainland instead of from northern beaches as MacCarthy and Martins have indicated.

(6) A very small amount of volcanic glass and a few species of heavy minerals occur well distributed over the region. The sources of all the volcanic glass and some of the heavy minerals are thought to be distant and that they are wind blown.

(7) Coal and ashes brought from outside sources by human agencies have been introduced into the sediments of Tortugas lagoon and, in smaller quantities, in a few other places.

(8) Pellets obtained from South Bight are tentatively classified as coprolites, largely on the basis of findings of H. B. Moore. However, it is felt that there may yet be another explanation advanced for the origin of some of them.

(9) Oolites may be forming today on the mud flats of western South Bight, but in most other places it appears that they have been derived from older formations. An origin similar to the one given by Mathews for Great Salt Lake oolites is favored. Today no such conditions are known to exist in the Floridian-Bahamian region as were prevalent while the Pleistocene Miami and Key West oolites were being deposited.

(10) In these sediments calcium and magnesium are combined almost entirely with carbonate, usually as calcite, but aragonite is not rare.

(11) The Ca/Mg ratio is determined largely by the percentage of magnesium-bearing coralline algæ present, and the lowest ratio is found in the sediments on the east side of Andros Island.

(12) The surface water at Tortugas is often super saturated with respect to calcium and carbonate ions during the summer months. Some chemical precipitation may occur, but the quantity of calcium carbonate thus brought down is insignificant in comparison with that derived from the calcareous tests of plants and animals.

(13) Aragonite needles are distributed over most of the area studied, but it appears that they have been washed from one or two local regions of precipitation. The greatest abundance is found in the shallow-water bottom muds from near the west entrance to South Bight.

Evidence that chemically precipitated calcium carbonate sediments are forming in west South Bight, on the Great Bahama Bank, and probably on some flats behind the Florida keys, is corroborated by the examination of a greater number of bottom specimens than has heretofore been studied in detail.

(14) On a quantitative basis about 66 per cent of the material is definitely known to be organic. In addition to this 7 per cent, unclassified fragments of tests, is almost certainly organic, and probably most of the silt and clay, totaling about 23 per cent is derived from organic remains. Only about 4 per cent is definitely known to be of inorganic origin.

B. Conclusions based on the series of 142 mechanical analyses of samples, presented as histograms, are as follows:

(1) Fine-grained calcareous muds show maximum quantities of silt and clay particles at and near the west end of South Bight where off-shore and other currents are absent or are very weak.

(2) Bottom deposits that usually have a low content of silt and clay occur at the east entrance to South Bight where there are strong tidal currents.

(3) Lagoon deposits have coarser-grained material on the slopes and tops of ridges; finer-grained sediments accumulate in the troughs. The protected flats on the lee, the west, side of Andros Island are generally covered by fine-grained sediments.

(4) The sediments of the northern part of the Florida reef tract, where the currents that flow along and among the keys are very variable, show all degrees of sorting, but usually the quantity of finer is less than that of the coarser particles.

C. The calcium carbonate sediments of the Bahamas and the Florida reef tract are compared with similar sediments from Murray Island, Australia; Pago Pago Harbor, Samoa; Funafuti Atoll; Maiao Island, South Pacific; and the calcareous beaches in high northern latitudes.

Aragonite needles, which occur abundantly in parts of the Bahamian and Floridian region, were not found at any other places except at Maiao Island, South Pacific. It appears that the physico-chemical conditions that lead to the precipitation of calcium carbonate are unusual in the ocean and occur in only a few restricted, relatively stagnant, shallow-water areas of the tropics and subtropics.

List of localities from which bottom samples were obtained

A. Bottom Samples Studied in Detail

Sample No.		Locality	Depth, feet
Florida	49	North Beacon east of Half Moon Shoal buoy	25
	50	1 mile east of 49	24
	51	1 mile east of 50, bottom lumpy, probably hard	23
	52	1 mile east of 51, lumpy bottom	26
	53	1 mile east of 52	20
	54	1 mile east of 53	23
	55	1 mile east of 54	23
	56	1 mile east of 55	23
	57	1 mile east of 56	28
	58	1 mile east of 57	23
	59	1 mile east of 58	14
	60	1 mile east of 59 (rock bottom very hard), 2.5 miles west of southwest corner of Marquesas	8
	62	1/4 mile south of south shore, southwest corner of Marquesas	6 or 7
	63	Marquesas Lagoon	2
	117	On bank 5 feet deep (water) east of east entrance north of channel into Marquesas	5
	120	Tortugas, Fort Jefferson Moat	36
	121	Tortugas, Bird Key harbor	36
	122	Tortugas, north end of Fort Jefferson wharf	18
	123	Tortugas, Fort Jefferson, north of coal shed	36
	124	Tortugas, Fort Jefferson wharf, off south coal shed	27
	126	Tortugas, Loggerhead Light bearing N 45° E, Spar buoy bearing S 10° E	12
	128	Tortugas	66
	129	Tortugas	54
	130	Tortugas	66
	131	Tortugas	57
	132	Tortugas	about 60
	133	Tortugas	60
	134	Tortugas	66
	134½	Tortugas	42-48
	136	Tortugas	66
	137	Tortugas	66
	138	Tortugas	63
	139	Tortugas	about 54
	140	Tortugas	about 54
	141	Tortugas	72
	143	Featherhead bank	about 48
	198	Between Cape Florida and Soldiers Key	9
	199	Between Cape Florida and Soldiers Key	7
	200	Between Cape Florida and Soldiers Key	12.5
	201	West end of Soldiers Key landing	about 9
	204	North side of Caesars Creek bank	9
	205	Off Rodriguez Key	12
	206	2 miles north of the F. E. C. Ry. terminal station, Key West	3

A. Bottom Samples Studied in Detail—(*Continued*)

Sample No.		Locality	Depth, feet
	206a	A little east of 206	6
	208	Tortugas	90-96
	209	Tortugas	114-120
	210	Tortugas	48-60
	212	Tortugas	48
	213	Tortugas	48
	214	Tortugas	60-66
Bahamas	144	South Bight, mangrove flat at Sandy Point	about 0.5
	145	South Bight, east entrance	about 1
	146	South Bight, east entrance	about 5
	147	South Bight, east entrance	about 8
	159	South Bight, east entrance	about 8
	162	South Bight, east entrance	about 8
	172	South Bight, east entrance	about 4
	175	South Bight, west entrance	about 2
	176a	South Bight, shore material, west entrance	0
	176b	South Bight, shore material, west entrance	0
	177	2 miles west of the mouth of South Bight (mud 2 feet deep)	6
	178	South Bight, west bank of entrance at west end (mud 7 feet deep)	water 2 ft.
	179	South Bight, shore material, southeast bank west end South Bight canal	0
	180	South Bight, shore material, southeast bank west end South Bight canal	0
	181	South Bight bar at head of South Bight canal	0-3
	182	South Bight, north side of Steamboat Cay (mud 2 feet deep)	water 3 ft.
	183	South Bight	about 3
	184a	Half-way between Mastick Point and Pye Point	6
	184b	Half-way between Mastick Point and Pye Point	6
	187	North side of Scott Key, off Conch Bay	4
	188	Off Cocoanut Point and Nicollstown Light	15
	189	Off Cocoanut Point and Nicollstown Light	10
	194	Beach sand, beach between north and south horns of Cocoanut Point	0
	195	Off Cocoanut Point at anchorage of yacht	7.5

B. Bottom Samples used for Comparison

Sample No.		Locality	Depth, feet
Florida	1	Junction of the two canals off mouth of Miami River	—
	3	Midway between main ship channel and West Point, Key Biscayne, between buoys 7 and 4	122
	10	Mainland north side of New Cut, Biscayne Bay	—
	11	North side of Norris Cut between Biscayne and ocean	—

B. Bottom Samples used for Comparison—(*Continued*)

Sample No.	Locality	Depth, feet
12	Northeastern corner of Key Biscayne	2.5
13	South of eastern end of Featherbed Bank	about 8
14	Lower Biscayne Bay, deepest portion, southwest of Sands Cut	12
15	West of Rubicon Key, off mouth of Caesar Creek, lower end of Biscayne Bay	11
16	0.5 mile west of inner end of Caesar Creek, lower end of Biscayne Bay	9
17	Off southern end, western side of Elliott Key, 50 feet offshore	2-4
18	Hawk Channel, off mouth of Broad Creek, 2 miles offshore	18
19	0.5 mile east of mouth of Angelfish Creek	12
20	Northern end of Card Sound, mouth of Broad Creek	about 9
21	Card Sound, west of Pumpkin Key, about 200 feet offshore	12
22	Card Sound, southwestern end, 0.5 mile west of Key Largo, and 0.5 mile north of spur at lower end of the Sound	about 10
23	Steamboat Creek, between Card and Barnes Sounds	12
24	Barnes Sound, near mouth of Steamboat Creek	about 12
25	Barnes Sound, center of the Sound	11
26	Blackwater Sound, upper end, off mouth of Jewfish Creek	12
29	Bay of Florida, entrance from Hoodoo Sound	9
30	Bay of Florida, shoal, 2 miles northeast of Pigeon Key	1
31	The material composing Pigeon Key	0
32	Off inner end of Tavernier Creek	7
33	Shoal southwest of Tavernier Creek about a mile off Long Island	1
34	On line between McGinty and Torrey Keys	6
35	1 mile northwest of Shell Key	9
36	Southwestern end of cut across bank south of Bowlegs Key	7
37	1.25 miles northeast of upper end of Long Key	6
38	1 mile west of north of western end of Long Key	6
39	1.25 miles north of eastern end of Grassy Key	12
40	1 mile north-northeast of Bamboo Key	9
41	0.75 mile north of eastern end of Stirrup Keys	9
43	0.5 mile southeast of Porpoise Key	4
44	0.25 mile west-northwest of northern end of No Name Key	9
45	Middle of channel between No Name and Big Pine Keys opposite middle of No Name Key	9
46	Eastern shore of No Name Key, opposite middle of island, 100 to 200 feet offshore	2.5
47	0.75 mile north of Bahia Honda Key	12

B. Bottom Samples used for Comparison—(*Continued*)

Sample No.	Locality	Depth, feet
68	Beach sand, east shore of Sands Key, Straits side	0
91	Mud flat, dredged material, north side of Loggerhead, east of Sugar Loaf Key	3-4
97	Near the obstruction buoy at southwest entrance to Fort Jefferson Channel, Tortugas	about 42
98a	Marquesas Lagoon, Florida, east side	1
98b	Marquesas Lagoon, east side	1
106	Marquesas Lagoon, east side. Core of bottom mud	Surface
110	Marquesas Lagoon, east side. Core of bottom mud	9
115	Marquesas Lagoon, east side. Core of bottom mud	Surface
Bahamas 6	Off eastern shore of Cat Key, south of passage between it and Cat Key	9
7	Off eastern shore of Gun Key, north of passage between it and Cat Key	about 12
8	Calcareous sand from shore, above tide, eastern face of Gun Key	0
9	Calcareous beach sand, western face of Cat Key	0
71	Great Bahama Bank, between Gun Key Light and Northwest Passage	about 14
79	South Bight, Andros Island	about 3
83	Shore material, west side of Andros Island	0
84	Tongue of the Ocean (Drew sta. No. 1)	(822 fms.)
85	Tongue of the Ocean (Drew sta. No. 2)	(825 fms.)
87	2 miles west mouth of South Bight, west side of Andros Island	about 3
88	2 miles west mouth of South Bight, west side of Andros Island	about 3
148	South Bight, east entrance	about 8
149	South Bight, east entrance	about 5
151	South Bight, east entrance	about 6.5
152	South Bight, east entrance	about 4
154	South Bight, east entrance	about 2
156	South Bight, east entrance	about 7
160-161	South Bight, east entrance	about 8
164	South Bight, east entrance	about 2
165	South Bight, east entrance	about 1
166	South Bight, east entrance	about 5
168	South Bight, east entrance	about 8
169	South Bight, east entrance	about 7.5
170	South Bight, east entrance	about 5
171	South Bight, east entrance	about 2.5
173	South Bight, east entrance	about 6
174	South Bight, east entrance	about 0.5
190	Off Cocoanut Point and Nicollstown Light	10
191	Off Cocoanut Point and Nicollstown Light	10
192	Off Cocoanut Point and Nicollstown Light	10
193	Off Cocoanut Point and Nicollstown Light	10

TABLE 12—*Mechanical analyses (A. Bottom samples studied in detail; B. Bottom samples used for comparison)*

A. BOTTOM SAMPLES STUDIED IN DETAIL

Number		Per cent retained on sieves					Silt	Clay
		2 – 1 mm.	1 – 0.5 mm.	0.5 – 0.25 mm.	0.25 – 0.1 mm.	0.1 – 0.05 mm.	0.05 – 0.005 mm.	0.005 – 0 mm.
Florida	49.....	16.2	29.4	25.6	27.7	0.1	0.1	1.2
	50.....	19.6	47.5	20.9	10.4	0.1	0.1	1.7
	51.....	17.6	49.3	20.6	10.4	0.3	0.5	1.6
	52.....	34.3	54.5	5.8	1.3	0.6	1.6	2.0
	53.....	40.4	39.4	16.3	2.7	0	0.4	1.3
	54.....	12.7	27.9	28.8	26.4	1.7	0.8	1.8
	55.....	2.1	18.7	46.6	32.1	0	0	0.4
	56.....	20.0	50.0	23.5	5.3	0	0.2	1.4
	57.....	29.4	33.0	13.4	20.3	1.0	1.1	1.6
	58.....	22.9	44.2	14.7	14.0	1.1	1.2	1.6
	59.....	14.8	68.4	13.7	1.4	0	0.2	1.1
	60.....	7.0	14.8	13.7	46.1	8.0	5.1	5.2
	62.....	7.8	19.4	13.8	29.3	13.2	8.2	8.2
	63.....	1.7	3.1	3.1	17.3	24.8	33.3	16.3
	117.....	0	17.9	17.5	38.6	7.8	6.0	11.7
	120.....	0	34.0	28.3	21.0	3.6	5.4	7.4
	121.....	0	0.2	0.4	6.6	23.8	56.2	12.6
	122.....	0	11.9	7.7	23.5	24.1	23.6	8.6
	123.....	0	9.6	10.5	32.3	22.0	17.9	7.7
	124.....	0	1.6	3.1	19.8	28.4	36.3	11.5
	126.....	0	5.8	30.4	61.3	0.5	0.4	2.4
	128.....	0	18.3	18.6	14.3	13.4	22.6	13.6
	129.....	0	3.7	5.9	19.5	18.5	37.9	15.0
	130.....	0	0	1.3	21.0	29.9	35.0	12.0
	131.....	0	1.2	1.6	23.7	29.0	33.9	10.9
	132.....	0	11.4	13.5	28.5	16.5	19.9	9.9
	133.....	0	13.1	12.6	21.8	12.0	28.8	12.1
	134.....	18.9	27.9	9.0	5.2	7.4	20.5	11.1
	134½....	0.8	1.8	3.0	26.6	29.8	28.8	9.1
	136.....	19.7	26.9	11.3	7.9	2.4	16.9	15.3
	137.....	12.4	30.8	15.8	9.2	4.9	16.0	11.3
	138.....	3.0	9.3	15.6	39.3	10.9	15.9	5.9
	139.....	8.5	9.4	7.8	25.3	20.1	21.1	7.4
	140.....	7.8	12.3	14.0	23.9	7.9	21.7	12.4
	141.....	28.6	30.1	7.1	4.4	4.1	14.0	12.1
	143.....	6.0	10.9	12.4	35.4	10.3	11.7	11.6
	198.....	0.7	4.0	19.5	74.0	0.2	0.1	1.0
	199.....	2.9	11.5	23.5	59.4	0.4	0.2	1.8
	200.....	1.4	4.1	13.6	71.5	2.9	1.7	4.6
	201.....	6.2	12.3	16.4	31.5	19.6	6.6	6.8
	204.....	5.7	9.2	7.9	27.6	14.1	17.2	17.4
	205.....	6.8	11.4	9.7	26.9	14.2	16.0	14.7
	206.....	10.2	21.4	19.2	20.1	4.4	9.9	14.2
	206a.....	8.6	16.6	7.2	23.0	9.4	14.8	20.6
	208.....	26.3	45.6	13.9	8.9	0.8	2.2	3.1

TABLE 12—*Mechanical analyses (A. Bottom samples studied in detail. B. Bottom samples used for comparison—(Continued)*

A. BOTTOM SAMPLES STUDIED IN DETAIL—(*Continued*)

Number		Per cent retained on sieves					Silt	Clay
		2 – 1 mm.	1 – 0.5 mm.	0.5 – 0.25 mm.	0.25 – 0.1 mm.	0.1 – 0.05 mm.	0.05 – 0.005 mm.	0.005 – 0 mm.
Florida, *Cont.*	209.....	0.4	0.6	0.5	19.3	21.9	47.3	9.2
	210.....	12.0	31.2	14.5	36.4	0.8	2.5	2.9
	212.....	12.4	29.2	13.0	31.0	2.8	6.8	4.9
	213.....	35.3	36.6	13.7	12.2	0.2	0.8	1.5
	214.....	3.8	8.0	4.1	33.4	18.4	24.1	8.2
Bahamas	144.....	10.0	23.2	18.5	32.4	9.2	4.5	2.8
	145.....	18.6	24.9	16.2	26.0	6.5	4.8	2.7
	146.....	16.6	36.0	22.0	15.0	4.5	3.5	2.0
	147.....	13.7	28.3	21.0	25.9	5.3	3.2	2.3
	159.....	22.4	29.1	4.9	41.6	0.1	0.5	1.3
	162.....	33.0	44.3	10.8	10.1	0.1	0.6	1.0
	172.....	9.0	42.2	34.9	12.8	0.2	0.4	0.4
	175.....	2.2	3.8	3.2	9.1	19.8	32.0	29.3
	176a.....	12.4	43.0	24.4	8.9	1.0	2.4	8.0
	176b.....	1.4	4.3	5.4	7.5	16.1	30.2	35.3
	177.....	1.6	4.5	5.0	6.3	12.2	31.1	40.1
	178.....	0.7	1.5	4.1	18.3	20.6	24.1	31.4
	179.....	0.5	1.5	1.2	5.8	19.3	31.1	40.2
	180.....	0.2	1.4	1.4	4.0	4.8	31.5	56.4
	181.....	1.3	4.1	6.6	10.6	14.0	27.6	34.8
	182.....	3.9	15.8	20.8	17.7	13.3	11.6	17.6
	183.....	0.4	4.6	6.2	13.9	12.9	24.9	37.5
	184a.....	3.0	33.5	29.7	31.5	1.1	0.2	1.0
	184b.....	6.3	19.5	17.4	34.4	13.3	7.1	2.5
	187.....	13.4	18.2	15.4	41.7	6.6	2.9	2.1
	188.....	0.2	7.6	41.0	50.4	0.1	0.2	0.3
	189.....	4.1	26.7	46.5	21.4	0.1	0.3	1.1
	194.....	0.1	12.8	28.4	58.1	0.1	0.1	0.7
	195.....	10.5	24.4	14.8	34.3	5.9	6.3	3.6

TABLE 12—*Mechanical analyses (A. Bottom samples studied in detail. B. Bottom samples used for comparison—(Continued)*

B. BOTTOM SAMPLES USED FOR COMPARISON

Number	Per cent retained on sieves					Silt and clay combined
	2 – 1 mm.	1 – 0.5 mm.	0.5 – 0.25 mm.	0.25 – 0.125 mm.	0.125 – 0.075 mm.	0.075 – 0 mm.
Florida 1	12.0	11.6	47.3	19.2	8.8	1.1
3	1.8	11.6	13.6	10.5	27.2	35.3
10	2.3	22.9	61.5	5.9	3.2	4.2
11	1.2	15.0	76.7	5.5	0.2	.....
12	4.6	11.0	45.7	32.5	6.4	.....
13	0.3	13.9	26.9	18.1	15.9	24.9
14	0.9	20.9	54.0	9.5	8.3	7.7
15	2.4	24.9	48.1	12.5	7.0	5.1
16	10.1	21.5	40.6	10.9	8.7	8.2
17	14.0	18.1	26.7	14.7	10.7	15.8
18	6.6	11.2	38.1	20.2	14.3	9.6
19	24.4	15.9	19.6	8.5	18.4	13.2
20	53.1	27.2	15.8	1.1	1.2	1.6
21	13.6	41.9	39.5	3.8	0.6	0.6
22	19.2	22.2	18.4	7.7	17.1	15.4
23	0	4.1	25.7	17.1	35.6	17.5
24	10.7	15.3	29.4	27.2	10.8	6.8
25	17.3	17.2	30.4	8.7	11.2	15.2
26	10.3	16.2	26.0	21.1	16.3	10.1
29	12.8	6.1	13.2	21.2	32.6	14.1
30	24.5	12.2	16.2	13.6	18.2	15.3
31	6.4	14.6	17.6	14.2	28.9	18.3
32	5.5	17.4	22.8	10.9	18.6	24.8
33	3.5	14.7	50.0	11.9	16.2	3.7
34	27.1	40.1	19.1	3.8	5.9	4.0
35	21.8	40.2	18.5	6.6	8.7	4.2
36	14.7	46.1	17.3	3.1	13.4	5.4
37	14.9	24.9	25.6	12.3	16.6	5.7
38	39.2	23.9	18.4	8.8	8.1	1.5
39	13.5	22.7	23.4	9.3	16.9	14.2
40	32.0	42.4	14.8	3.4	4.2	3.2
41	27.6	28.4	14.4	7.1	6.7	14.8
43	11.6	54.2	25.6	1.9	2.0	4.7
44	17.8	38.3	16.4	13.8	9.2	4.5
45	19.6	33.2	21.3	10.2	12.6	3.1
46	27.9	33.4	22.4	5.9	5.6	4.8
47	19.4	25.1	24.8	15.2	11.7	3.8

Number	Per cent retained on sieves					Silt	Clay
	2 – 1 mm.	1 – 0.5 mm.	0.5 – 0.25 mm.	0.25 – 0.1 mm.	0.1 – 0.05 mm.	0.05 – 0.005 mm.	0.005 – 0 mm.
Florida 68	47.2	46.1	1.8	1.1	0.3	1.9	2.4
91	1.7	6.3	4.1	5.9	16.6	27.9	36.6
97	1.7	2.2	2.3	18.7	37.9	31.0	6.9
98a	4.0	6.4	4.0	19.8	25.6	27.4	12.6
98b	2.8	6.9	4.5	19.8	24.2	22.0	19.9
106	15.9	24.1	10.6	9.1	6.6	17.3	16.4
110	1.9	4.3	4.7	18.2	16.5	26.5	28.1
115	0	17.4	14.0	32.0	13.0	9.7	14.3

TABLE 12—*Mechanical analyses* (*A. Bottom samples studied in detail. B. Bottom samples used for comparison—(Continued)*
B. BOTTOM SAMPLES USED FOR COMPARISON—(*Continued*)

Number	Per cent retained on sieves					Silt and clay combined
	2 – 1 mm.	1 – 0.5 mm.	0.5 – 0.25 mm.	0.25 – 0.125 mm.	0.125 – 0.075 mm.	0.075 – 0 mm.
Bahamas 6.....	6.7	12.2	35.9	9.9	19.4	15.9
7.....	3.0	12.9	21.6	11.7	30.0	20.8
8.....	0.4	7.3	44.2	24.2	10.7	13.2
9.....	0.5	36.1	62.7	0.6	0.1	.....

Number	Per cent retained on sieves					Silt	Clay
	2 – 1 mm.	1 – 0.5 mm.	0.5 – 0.25 mm.	0.25 – 0.1 mm.	0.1 – 0.05 mm.	0.05 – 0.005 mm.	0.005 – 0 mm.
Bahamas 71.....	2.1	16.3	38.2	40.1	1.7	0.3	2.0
79.....	1.2	5.0	9.1	16.5	10.6	24.5	33.1
83.....	9.4	9.6	4.0	8.0	14.4	29.1	26.3
84.....	.4	2.2	4.5	11.3	15.5	45.2	20.5
85.....	.1	.5	.6	2.3	7.1	55.3	34.6
87.....	1.0	1.6	1.7	10.2	25.1	25.8	35.4
88.....	0.6	1.9	4.3	16.1	27.9	19.5	30.3
148.....	6.2	19.7	16.5	29.7	18.6	6.5	2.2
149.....	9.4	14.2	17.8	49.7	3.9	2.8	2.1
151.....	10.9	14.4	14.2	53.3	3.4	1.6	2.1
152.....	5.1	12.2	16.6	61.2	2.0	1.2	1.6
154.....	1.2	3.0	14.5	79.9	.3	.2	1.2
156.....	5.9	21.5	19.0	43.7	3.9	3.3	2.8
160–161.....	55.4	22.0	5.1	15.7	.1	.8	.6
164.....	10.3	29.7	41.4	16.8	.1	.3	1.3
165.....	11.9	41.9	23.7	19.5	.5	1.2	1.4
166.....	1.2	28.2	50.5	19.4	.1	.1	.6
168.....	9.6	20.6	29.1	39.9	.2	.2	.8
169.....	9.3	25.9	26.1	26.9	7.0	3.2	2.0
170.....	9.5	18.0	13.4	33.5	14.4	9.1	2.5
171.....	8.2	33.5	34.6	22.6	.3	.3	.6
173.....	14.2	24.6	20.0	35.2	3.2	1.7	1.2
174.....	9.6	40.0	30.4	16.2	1.6	.7	1.3
190.....	.2	2.0	9.2	85.5	2.5	.1	1.4
191.....	2.8	13.5	27.7	53.2	1.2	.4	1.4
192.....	6.9	29.5	29.2	31.9	.9	.6	1.1
193.....	5.6	15.0	16.5	55.8	5.2	.7	1.2

TABLE 13—*Microscopical analyses*
A. FLORIDA SAMPLES 49 TO 63

Organic Constituents	Sample No.													
	49	50	51	52	53	54	55	56	57	58	59	60	62	63
Algæ	37.6	33.5	32.6	37.6	32.6	29.6	34.8	39.0	36.9	38.8	34.4	27.4	38.4	12.5
Coralline	A	A	A	A	A	A	A	A	A	A	A	VC	A	C
Halimeda	A	A	A	A	A	A	A	A	A	A	A	A	A	VC
Bryozoa	0.5	tr.	.....	.....	.....	0.6	tr.	tr.	0.6	.....	0.2	.....	.....	0.3
Coral	7.9	9.9	6.9	9.7	7.9	11.7	6.2	11.1	12.6	10.3	12.8	9.4	5.9	3.2
Crustacea	0.5	.....	0.5	1.0	0.8	1.4	0.8	0.8	tr.	0.2	0.4	0.9	1.5	4.0
Decapod	.....	.....	.....	.....	R	R	R	R	.....	R	R	R	R	C
Ostracod	R	.....	R	FC	.....	FC	R	R	R	.....	.....	R	FC	C
Fish otoliths, teeth	.....	.....	.....	.....	.....	.....	tr.	tr.	.....	.....	.....	.....	.....	.....
Foraminifera	9.9	8.9	8.9	7.7	9.9	8.8	10.0	7.8	7.4	10.7	10.8	18.8	11.7	12.0
Amphistegina	.....	.....	.....	.....	.....	R	.....	.....	.....	.....	.....	.....	.....	.....
Anomalina	.....	R	R	R	.....	.....	.....	.....	R	.....	.....	R	FC	FC
Archaias	FC	FC	FC	FC	FC	C	FC	FC	C	VC	VC	VC	VC	C
Articulina	.....	R	.....	FC	.....	.....	.....	.....	.....	.....	.....	R	R	.....
Asterigerina	R	.....	R	R	.....	FC	.....	.....	R	.....	.....	.....	.....	.....
Bolivina	.....	.....	.....	.....	.....	.....	.....	.....	.....	R	.....	.....	.....	R
Borelis	.....	R	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Cibicides	.....	.....	.....	.....	.....	R	.....	.....	.....	.....	.....	.....	R	R
Clavulina	.....	.....	.....	.....	.....	.....	.....	.....	R	.....	.....	.....	.....	.....
Discorbis	.....	.....	.....	R	.....	.....	.....	.....	.....	.....	.....	.....	R	FC
Elphidium	.....	.....	.....	.....	.....	R	.....	.....	.....	.....	.....	.....	R	.....
Lagena	.....	.....	.....	R	.....	.....	.....	.....	.....	R	.....	.....	.....	.....
Nonion	.....	.....	.....	R	.....	R	.....	.....	.....	.....	R	R	R	FC
Peneroplis	FC	.....	.....	.....	.....	R	R	.....	R	.....	.....	FC	.....	R
Planorbulina	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	R	.....	.....
Pyrgo	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	R	R
Quinqueloculina	FC	FC	FC	C	FC	FC	C	FC	FC	R	.....	FC	C	VC
Rotalia	FC	R	FC	FC	FC	FC	FC	FC	FC	R	FC	C	FC	C
Sorites	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	R
Spiroloculina	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	R
Textularia	.....	R	.....	FC	.....	.....	.....	.....	.....	.....	R	R	.....	.....
Valvulina	.....	R	.....	.....	.....	.....	.....	.....	.....	.....	.....	R	.....	R

TABLE 13—*Microscopical analyses—(Continued)*
A. FLORIDA SAMPLES 49 TO 63—*(Continued)*

Organic Constituents	Sample No.													
	49	50	51	52	53	54	55	56	57	58	59	60	62	63
Mollusk	25.7	31.1	28.4	24.6	33.0	25.3	32.9	26.6	23.3	23.3	22.0	21.5	14.2	9.3
Gastropods	A	A	A	A	A	A	A	A	A	A	A	A	C	C
Pelecypods	VC	A	A	A	A	A	A	A	VC	VC	VC	VC	C	FC
Spicules, fragments	4.2	3.2	6.9	5.8	2.2	7.2	4.0	2.0	4.0	4.9	6.5	6.3	4.6	2.3
Alcyonarian	FC	FC	C	C	FC	C	R	R	FC	C	FC	FC	C	FC
Echinoid	FC	R	R	FC	R	R	R	.....	R	FC	R	FC	R	R
Sponge	.....	.....	.....	R	.....	FC	R	.....	.....	.....	R	FC	FC	FC
Tunicate	.....	.....	.....	.....	.....	FC	.....	R	.....	.....	FC	.....	.....	R
Worm tubes	tr.	.....	0.2	1.5	1.2	3.9	1.6	1.8	2.0	0.2	0.6	0.9	1.7	1.4
Pellets	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	2.0
Inorganic and questionable Organic Constituents														
Minerals	1.0	1.0	0.6	2.1	1.0	0.8	0.8	1.8	4.0	1.2	4.1	0.7	0.5	0.3
Carbonaceous grains	R	R	.....	FC	FC	.....	FC	FC	C	FC	FC	.....	R	.....
Coal, cinders	R	.....	.....	.....	R	.....	.....	.....	.....	.....	.....	.....	.....	.....
Glauconite	R	.....	R	.....	.....	R	.....	R	R	.....	R	.....	.....	.....
Gypsum	R	R	R	R	R	R	.....	R	R	.....	R	.....	.....	.....
Mica	R	.....	.....	.....	.....	.....	.....	R	.....	.....	R	R	R	.....
Quartz	R	.....	R	R	R	R	R	.....	R	R	C	R	R	R
Quartzite	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	FC	R	.....	.....
$CaCO_3$ fragments	10.3	9.9	9.9	6.8	8.9	7.8	9.0	5.0	6.3	7.3	5.7	3.2	5.0	2.5
Oolites	1.2	1.4	3.0	0.0	1.0	0.4	tr.	3.0	0.4	0.4	0.6	.....	0.5	0.2
Silt	0.1	0.1	0.5	1.6	0.4	0.8	.....	0.2	1.1	1.2	0.2	5.1	8.2	33.3
Clay	1.2	1.7	1.6	2.0	1.3	1.8	0.4	1.4	1.6	1.6	1.1	5.2	8.2	16.3
Total	100.1	100.7	100.0	100.4	100.2	100.1	100.5	100.5	100.2	100.1	99.4	99.4	100.4	99.6

TABLE 13—*Microscopical analyses—(Continued)* B. FLORIDA SAMPLES 117 TO 134½.

Organic Constituents	Sample No.														
	117	120	121	122	123	124	126	128	129	130	131	132	133	134	134½
Algæ	47.6	31.4	11.2	19.0	19.7	25.8	39.2	19.8	13.1	14.0	15.3	17.5	11.1	16.4	18.6
Coralline	A	A	A	A	A	A	A	C	C	VC	A	A	C	A	A
Halimeda	A	A	VC	VC	A	A	A	VC	VC	VC	A	A	C	VC	A
Bryozoa	.....	0.9	0.2	0.5	0.6	tr.	tr.	0.3	0.5	1.2	0.8	tr.	0.6	1.0	0.5
Coral	5.2	15.2	2.9	14.1	21.6	8.6	26.5	18.1	8.9	2.7	5.1	17.5	11.2	11.6	3.1
Crustacea	0.8	0.9	1.6	1.0	1.0	2.0	0.8	1.0	2.3	2.1	2.8	2.9	2.7	2.9	4.4
Decapod	R	R	.....	R	.....	R	.....	.....	R	R	FC	C	FC	R	C
Ostracod	R	R	C	R	R	FC	R	R	C	C	C	R	FC	FC	FC
Fish otoliths, teeth	.....	.....	.....	.....	tr.	.....	.....	.....	.....	.....	.....	tr.	.....	.....	.....
Foraminifera	8.2	6.2	3.1	4.7	8.0	5.9	8.5	6.5	8.9	12.6	8.3	6.7	4.8	4.8	3.3
Ammobaculites	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	R	R	R	R	R
Amphistegina	.....	R	R	.....	R	.....	R	R	FC	.....	R	R	R	.....	.....
Anomalina	R	FC	R	R	.....	.....	.....	.....	R	FC	FC	R	R	R	FC
Archaias	VC	FC	FC	FC	FC	FC	C	FC	R	R	FC	R	R	FC	FC
Articulina	.....	.....	R	R	.....	R	.....	R	R	.....	R	.....	.....	.....	R
Asterigerina	.....	.....	.....	.....	.....	.....	R	R	R	R	FC	FC	FC	.....	R
Bolivina	R	R	.....	.....	R	.....	.....	FC	FC	R	R	R	.....	.....	R
Borelis	.....	.....	.....	.....	.....	.....	.....	R	.....	.....	.....	.....	.....	.....	.....
Bulimina	.....	.....	.....	.....	R	.....	.....	.....	R	R	R	.....	.....	.....	.....
Clavulina	.....	.....	.....	.....	R	R	.....	.....	R	.....	R	.....	.....	.....	.....
Cymbaloporetta	.....	.....	R	.....	.....	R	R	R	FC	FC	R	R	R	R	R
Discorbis	.....	.....	.....	.....	.....	.....	.....	.....	.....	R	R	R	R	.....	.....
Elphidium	.....	.....	.....	R	.....	.....	R	FC	FC	C	FC	FC	FC	FC	FC
Globigerina	.....	.....	.....	.....	.....	.....	.....	.....	R	.....	.....	.....	.....	R	R
Homotrema	.....	.....	.....	.....	R	R	R	.....	.....	.....	.....	.....	.....	.....	.....
Lagena	.....	.....	.....	.....	FC	.....	.....	.....	.....	.....	.....	.....	R	R	R
Nodosaria	.....	.....	.....	.....	R	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Nonion	R	.....	.....	R	FC	R	.....	FC	FC	R	.....	.....	.....	.....	FC
Peneroplis	.....	.....	.....	R	R	R	R	.....	FC	R	FC	FC	FC	R	R
Planorbulina	.....	R	.....	.....	R	R	.....	R	.....	R	R	.....	.....	.....	R
Pyrgo	.....	.....	.....	R	.....	.....	R	R	R	.....	C	.....	R	R	R
Quinqueloculina	FC	FC	R	FC	FC	C	C	C	A	A	VC	C	VC	C	C
Reussella	.....	.....	.....	R	.....	.....	.....	.....	R	.....	.....	.....	.....	.....	.....
Rotalia	FC	R	.....	.....	R	FC	FC	R	R	R	R	R	.....	R	.....
Sorites	.....	.....	.....	.....	.....	R	R	.....	FC	FC	FC	R	R	.....	.....

TABLE 13—*Microscopical analyses*—(*Continued*) B. FLORIDA SAMPLES 117 TO 134½—(*Continued*)

Organic Constituents	Sample No.														
	117	120	121	122	123	124	126	128	129	130	131	132	133	134	134½
Spiroloculina	.....	.....	.....	.....	.....	.....	.....	.....	FC	.....	.....	.....	.....	R	R
Textularia	.....	.....	.....	.....	.....	R	R	R	.....	C	FC	R	FC	R	R
Valvulina	.....	.....	.....	.....	.....	R	.....	.....	.....	R	R	.....	.....	.....	.....
Mollusk	8.2	17.3	5.6	7.6	10.2	5.3	12.3	9.7	7.1	16.0	15.3	14.9	17.2	18.5	19.8
Gastropods	C	C	VC	FC	VC	C	A	C	VC	A	VC	VC	A	A	A
Pelecypods	C	VC	FC	FC	C	C	C	C	VC	A	A	VC	A	A	VC
Spicules, fragments	3.3	3.0	2.7	3.4	4.7	2.1	7.4	2.7	3.6	4.4	4.6	5.8	3.1	2.5	4.6
Alcyonarian	R	R	FC	FC	FC	FC	C	FC	C	C	C	C	FC	FC	C
Echinoid	R	R	R	R	R	R	R	R	R	R	R	R	.....	.....	R
Sponge	.....	R	FC	C	C	R	FC	FC	FC	C	FC	FC	FC	FC	FC
Tunicate	R	R	.....	.....	FC	R	R	R	R	R	FC	FC	R	R	FC
Worm tubes	1.6	0.9	0.3	0.8	0.2	0.5	1.5	0.1	0.6	0.4	tr.	0.2	0.8	2.3	1.0
Pellets	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	2.8	.....	.....	.....	.....
Inorganic and Questionable Organic Constituents															
Minerals	0.2	5.9	0.7	10.9	2.6	1.2	tr.	0.3	0.4	tr.	tr.	0.7	3.0	2.7	0.5
Carbonaceous grains	R	FC	.....	.....	.....	.....	.....	C	C	.....	.....	C	VC	C	FC
Chert	.....	FC	.....	R	R	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Coal, cinders	.....	VC	FC	A	VC	R	.....	R	R	R	R	.....	.....	.....	.....
Garnet	.....	R	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Gypsum	.....	.....	.....	.....	R	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Hematite	.....	R	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Limonite	.....	.....	.....	R	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Magnetite	.....	R	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Mica	.....	.....	R	R	R	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Olivine	.....	R	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Quartz	R	C	R	FC	.....	R	R	.....	R	.....	.....	.....	R	R	R
Volcanic ash, glass	.....	R	.....	.....	.....	FC	R	.....	.....	.....	.....	.....	R	R	.....
$CaCO_3$ fragments	6.6	5.2	2.3	5.2	6.5	1.2	1.7	6.5	2.0	.....	0.6	2.9	4.5	4.8	6.0
Oolites	0.2	.....	.....	.....	.....	.....	.....	tr.	.....	.....	.....	.....	0.5	1.4	tr.
Silt	6.0	5.4	56.2	23.6	17.9	36.3	0.4	22.6	37.9	35.0	33.9	19.9	28.8	20.5	28.8
Clay	11.6	7.4	12.6	8.6	7.7	11.5	2.4	13.6	15.0	12.0	10.9	9.9	12.1	11.1	9.1
Total	99.5	99.7	99.4	99.4	100.7	100.4	100.7	101.2	100.3	100.4	99.6	98.9	100.4	100.5	99.7

TABLE 13—*Microscopical analyses—(Continued)* C. FLORIDA SAMPLES 136 TO 206a.

Organic Constituents	Sample No.														
	136	137	138	139	140	141	143	198	199	200	201	204	205	206	206a
Algæ	24.6	18.0	22.6	19.9	20.4	22.3	22.4	18.5	24.8	39.4	27.6	20.0	26.2	37.7	16.2
Coralline	A	A	A	A	A	A	A	A	A	FC	FC	VC	C	C	VC
Halimeda	A	A	A	VC	A	A	VC	A	A	A	A	A	A	A	A
Bryozoa	1.2	1.9	0.6	1.4	1.6	0.6	.....	.....	.....	.....	.....	0.2	0.3	.....	.....
Coral	8.6	13.2	11.7	10.0	10.5	10.4	4.7	2.0	1.6	2.8	12.0	2.5	6.4	9.0	3.2
Crustacea	0.8	0.9	2.5	2.4	1.6	2.8	2.4	tr.	0.2	0.9	1.4	1.1	1.6	0.7	0.4
Decapod	R	R	R	R	R	R	R	.....	R	R	R	R	R	R	R
Ostracod	R	R	FC	R	R	FC	C	R	.....	FC	FC	C	FC	FC	FC
Fish otoliths, teeth	.....	tr.	tr.	tr.	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Foraminifera	3.0	2.8	5.5	4.7	4.0	4.9	15.2	12.2	7.6	9.4	6.9	17.4	11.7	12.1	18.2
Ammobaculites	R	R	R	R	R	.....	.....	.....	.....	.....	.....	.....	R	R	R
Amphistegina	R	R	R	R	FC	.....	.....	.....	.....	.....	.....	.....	.....	R	.....
Anomalina	R	R	FC	R	.....	R	R	.....	.....	.....	R	FC	.....	R	R
Archaias	.....	R	FC	FC	R	R	C	VC	C	C	FC	VC	VC	VC	VC
Articulina	.....	.....	R	R	.....	.....	.....	.....	.....	.....	.....	FC	.....	.....	.....
Asterigerina	R	.....	FC	.....	R	R	.....	R	.....	.....	.....	.....	.....	.....	.....
Bolivina	R	R	R	R	.....	.....	.....	.....	.....	.....	FC	FC	.....	R	R
Borelis	.....	R	.....	R	.....	R	.....	.....	.....	R	.....	.....	.....	.....	.....
Bulimina	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	R	.....
Cibicides	.....	FC	.....	.....	R	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Clavulina	.....	R	.....	.....	R	.....	FC	.....	.....	R	.....	R	R	R	.....
Cymbaloporetta	.....	.....	R	R	R	.....	.....	.....	.....	.....	.....	R	.....	.....	.....
Discorbis	.....	.....	R	R	.....	R	C	.....	.....	R	R	FC	FC	R	FC
Elphidium	R	.....	FC	FC	FC	R	R	.....	R	R	.....	.....	R	.....	R
Globigerina	.....	.....	R	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Lagena	.....	.....	FC	.....	.....	R	R	.....	.....	.....	.....	R	.....	FC	R
Nodosaria	R	R	R	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Nonion	.....	R	.....	.....	.....	.....	FC	.....	R	R	FC	FC	FC	R	FC
Peneroplis	.....	.....	R	R	R	.....	R	.....	R	R	.....	FC	R	R	R
Planorbulina	.....	.....	.....	.....	.....	R	.....	.....	.....	.....	.....	.....	.....	.....	.....
Pyrgo	R	.....	R	.....	.....	.....	FC	.....	.....	FC	R	.....	FC	FC	.....
Quinqueloculina	FC	FC	C	C	C	FC	VC	C	FC	VC	FC	A	VC	C	C
Rotalia	.....	.....	R	.....	R	.....	R	.....	.....	.....	R	R	R	.....	.....
Sorites	.....	.....	R	.....	R	.....	R	R	.....	.....	.....	.....	.....	R	R
Spiroloculina	R	.....	.....	R	.....	.....	R	.....	.....	R	.....	.....	R	.....	.....

TABLE 13—*Microscopical analyses*—(*Continued*) C. FLORIDA SAMPLES 136 TO 206a—(*Continued*)

Organic Constituents	Sample No.														
	136	137	138	139	140	141	143	198	199	200	201	204	205	206	206a
Textularia	.....	.....	FC	R	FC	.....	FC	.....	.....	.....	.....	.....	.....	.....	.....
Valvulina	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	R	R	R	.....	.....
Mollusk	13.0	20.8	20.7	18.5	14.9	18.6	12.9	11.4	12.9	12.5	10.0	15.5	11.7	7.8	18.2
Gastropods	.....	A	A	A	VC	A	VC	VC	VC	VC	VC	A	C	C	A
Pelecypods	.....	A	A	A	A	A	C	VC	VC	VC	VC	A	VC	C	VC
Pteropods	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	FC	FC	R	FC
Radiolarians	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	tr.	tr.	.....	tr.	tr.
Spicules, fragments	3.7	5.0	3.9	4.3	3.6	5.9	1.1	4.1	2.2	1.1	4.7	0.7	0.7	1.4	1.3
Alcyonarian	.....	C	FC	FC	FC	C	R	C	FC	FC	C	R	R	FC	FC
Echinoid	.....	R	FC	FC	R	R	R	R	FC	R	R	R	R	R	R
Sponge	.....	FC	FC	FC	R	C	R	FC	R	R	FC	R	R	FC	FC
Tunicate	.....	FC	R	FC	FC	FC	FC	.....	.....	R	FC	R	R	R	R
Worm tubes	3.0	5.9	2.2	2.8	3.3	3.9	1.8	tr.	2.5	1.5	0.8	1.4	2.3	0.6	0.6
Pellets	.....	.....	.....	.....	.....	.....	0.6	.....	.....	.....	.....	.....	0.1	.....	.....
Inorganic and Questionable Organic Constituents															
Minerals	3.8	1.8	2.8	2.1	2.8	0.7	11.7	47.2	39.0	17.8	16.8	0.3	tr.	tr.	0.3
Carbonaceous grains	C	R	FC	FC	FC	R	.....	FC	R	.....	.....	.....	.....	.....	FC
Coal, cinders	.....	FC	.....	.....	.....	R	.....	R	.....	.....	R	.....	.....	.....	R
Epidote	.....	.....	.....	.....	.....	.....	.....	R	R	R	R	.....	.....	.....	.....
Garnet	.....	.....	.....	.....	.....	.....	.....	.....	R	.....	.....	.....	.....	R	.....
Gypsum	R	R	R	R	R	R	.....	R	.....	.....	R	.....	.....	.....	.....
Jasper	.....	.....	.....	.....	.....	.....	R	.....	.....	.....	.....	.....	.....	.....	.....
Limonite	.....	.....	.....	.....	.....	.....	R	.....	.....	.....	.....	.....	.....	.....	.....
Magnetite	.....	.....	.....	.....	.....	.....	.....	FC	FC	R	R	.....	.....	.....	R
Mica	.....	R	R	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Quartz	R	R	.....	R	R	.....	A	A	A	A	A	FC	R	R	R
Volcanic ash, glass	.....	.....	.....	R	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
$CaCO_3$ fragments	5.2	2.9	5.5	4.7	3.0	4.5	3.2	2.0	5.9	7.8	5.7	4.1	6.2	6.0	6.5
Oolites	1.0	0.1	tr.	.....	tr.	tr.	tr.	1.0	1.0	0.4	0.2	1.2	1.0	.....	.....
Silt	16.9	16.0	15.9	21.1	21.7	14.0	11.7	0.1	0.2	1.7	6.6	17.2	16.0	9.9	14.9
Clay	15.3	11.3	5.9	7.4	12.4	12.1	11.6	1.0	1.8	4.6	6.8	17.4	14.7	14.2	20.6
Total	100.1	100.6	99.8	99.3	99.8	100.7	99.3	99.5	99.7	99.9	100.4	99.0	98.9	99.4	100.4

TABLE 13—*Microscopical analyses—(Continued)*
D. FLORIDA SAMPLES 208 TO 214

Organic Constituents	Sample No.					
	208	209	210	212	213	214
Algæ	24.8	2.6	17.5	23.0	23.9	19.0
Coralline	A	R	A	VC	A	VC
Halimeda	A	FC	A	A	A	A
Bryozoa	1.0	0.3	0.8	0.9	1.0	0.8
Coral	8.0	.....	18.0	8.0	24.1	2.3
Crustacea	1.3	2.8	1.7	1.0	1.0	2.7
Decapod	R	.....	C	R	R	FC
Ostracod	FC	FC	R	FC	R	FC
Fish otoliths, teeth	.....	.....	tr.	.....	.....	.....
Foraminifera	11.1	6.4	17.1	17.7	7.5	9.2
Ammobaculites	R	R	R	R	.....	R
Amphistegina	R	.....	R	R	R	.....
Anomalina	R	R	.....	.....	R	FC
Archaias	C	R	C	VC	FC	C
Articulina	R	R	R	R	R	R
Asterigerina	R	R	FC	FC	FC	FC
Bolivina	.....	R	.....	.....	.....	.....
Bulimina	R	.....	.....	.....	.....	.....
Clavulina	.....	.....	.....	R	.....	.....
Discorbis	R	R	R	R	.....	.....
Elphidium	.....	R	.....	R	.....	.....
Globigerina	.....	R	.....	.....	.....	.....
Lagena	R	R	.....	R	.....	R
Nodosaria	R	R	.....	.....	.....	.....
Nonion	R	R	R	R	.....	R
Peneroplis	FC	R	C	FC	.....	FC
Planorbulina	.....	.....	R	.....	R	.....
Pyrgo	.....	R	R	R	.....	R
Quinqueloculina	C	FC	C	A	FC	VC
Reussella	.....	.....	.....	.....	R	.....
Rotalia	R	.....	R	R	R	.....
Sorites	R	R	.....	C	.....	FC
Spiroloculina	.....	FC	.....	FC	.....	.....
Textularia	R	R	R	R	R	R
Mollusk	33.4	15.8	22.8	17.7	20.4	17.3
Gastropods	A	VC	A	VC	A	VC
Pelecypods	A	A	A	A	A	VC
Pteropods	FC	C	.....	FC	R	R
Spicules, fragments	4.6	5.5	9.5	11.9	10.4	7.9
Alcyonarian	FC	FC	VC	VC	VC	C
Echinoid	R	R	FC	C	FC	FC
Sponge	FC	C	R	FC	FC	FC
Tunicate	C	C	.....	C	C	C
Worm tubes	4.8	tr.	0.4	2.3	1.6	2.0
Pellets	.....	3.0	.....	.....	.....	.....
Inorganic and Questionable Organic Constituents						
Minerals	1.0	0.7	0.4	0.9	tr.	tr.
Carbonaceous grains	FC	.....	.....	FC	.....	.....
Coal, cinders	FC	.....	R	.....	.....	.....
Glauconite	.....	.....	.....	R	.....	.....
Gypsum	R	.....	R	R	.....	.....
Mica	.....	FC	R	.....	R	R
$CaCO_3$ fragments	4.8	5.6	6.6	5.3	7.8	6.5
Oolites	0.8	.....	.....	.....	.....	.....
Silt	2.2	47.3	2.5	6.8	0.8	24.1
Clay	3.1	9.2	2.9	4.9	1.5	8.2
Total	100.9	99.2	100.2	100.4	100.0	100.0

TABLE 13—*Microscopical analyses*—(*Continued*)
E. BAHAMA SAMPLES 144 TO 181

Organic Constituents	Sample No.														
	144	145	146	147	159	162	172	175	176a	176b	177	178	179	180	181
Algæ	15.9	16.6	25.4	33.0	30.0	27.0	19.4	4.7	26.9	0.8	1.2	2.5	1.4	tr.	1.7
Coralline	A	A	A	A	A	A	A	C	A	R	R	R	R	.....	R
Halimeda	FC	C	VC	A	FC	VC	C	C	C	R	FC	FC	R	R	R
Bryozoa	tr.	tr.	tr.	tr.	.....	0.2	.....	.....	.....	.....	.....	.....	.....	.....	.....
Coral	20.5	8.3	9.4	13.2	11.0	20.6	31.7	0.2	.....	.....	.....	tr.	.....	.....	.....
Crustacea	0.9	1.1	1.5	1.3	1.0	tr.	.....	2.0	0.9	0.3	0.9	0.8	1.7	tr.	1.2
Decapod	.....	.....	R	.....	R	.....	.....	.....	.....	R	FC	R	C	R	.....
Ostracod	FC	FC	FC	FC	FC	R	.....	C	R	R	FC	FC	FC	R	R
Fish otoliths, teeth	.....	.....	.....	tr.	.....	.....	tr.	.....	.....	.....	.....	.....	.....	.....	.....
Foraminifera	24.3	31.4	32.0	25.4	23.2	27.0	16.8	11.4	22.4	1.9	8.3	12.2	7.1	0.4	15.4
Amphistegina	.....	.....	.....	.....	.....	.....	.....	FC	.....	.....	.....	.....	.....	.....	.....
Anomalina	FC	FC	C	R	FC	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Archaias	A	A	VC	A	A	VC	VC	VC	VC	R	C	VC	VC	R	VC
Articulina	R	R	R	R	R	R	.....	.....	.....	.....	R	.....	.....	.....	.....
Asterigerina	.....	.....	FC	C	C	FC	R	.....	.....	.....	.....	.....	R	.....	R
Bolivina	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	R	R	R
Borelis	.....	R	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Bulimina	.....	.....	.....	.....	.....	.....	.....	.....	.....	R	R	.....	.....	.....	.....
Cibicides	.....	.....	.....	.....	.....	.....	.....	FC	.....	.....	.....	.....	.....	.....	.....
Clavulina	FC	FC	R	C	R	.....	R	.....	R	.....	.....	.....	R	.....	R
Cymbaloporetta	FC	.....	R	R	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Discorbis	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	R	R	.....	R	R
Elphidium	.....	.....	.....	.....	.....	.....	.....	R	FC	FC	R	R	R	.....	FC
Lagena	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	FC	.....	.....	.....
Nodosaria	FC	.....	.....	.....	.....	.....	R	.....	.....	.....	.....	.....	.....	.....	.....
Nonion	R	FC	R	R	R	.....	.....	R	FC	.....	.....	.....	R	.....	.....
Peneroplis	C	C	FC	C	FC	FC	FC	C	FC	.....	C	C	R	.....	FC
Planorbulina	.....	.....	R	FC	R	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Pyrgo	.....	.....	.....	.....	.....	.....	.....	FC	.....	R	.....	R	R	.....	.....
Quinqueloculina	VC	A	A	A	A	FC	C	C	FC	FC	C	FC	FC	FC	VC
Rotalia	FC	R	FC	FC	FC	.....	C	.....	.....	.....	.....	.....	.....	R	.....
Sorites	R	R	R	FC	R	R	.....	.....	.....	R	.....	R	.....	.....	.....

TABLE 13—*Microscopical analyses—(Continued)*
E. BAHAMA SAMPLES 144 TO 181—*(Continued)*

Organic Constituents	Sample No.														
	144	145	146	147	159	162	172	175	176a	176b	177	178	179	180	181
Verneuilina	FC	FC	R	.....	.....	R	.....	.....	.....	.....	.....	.....	.....	.....	.....
Valvulina	C	FC	R	R	.....	.....	.....	.....	.....	.....	.....	R	.....	.....	R
Mollusk	9.3	11.1	14.1	11.6	14.7	10.3	12.9	13.4	24.2	1.0	3.0	10.4	7.4	1.5	5.1
Gastropods	A	A	VC	A	A	VC	VC	A	A	R	R	C	C	FC	C
Pelecypods	FC	C	VC	FC	C	C	VC	FC	A	R	FC	VC	C	FC	C
Pteropods	.....	.....	.....	R	.....	.....	.....	.....	.....	R	.....	.....	.....	.....	.....
Spicules, fragments	2.3	2.8	1.9	2.5	2.9	2.8	4.0	tr.	tr.	tr.	tr.	0.2	tr.	tr.	0.5
Alcyonarian	FC	FC	FC	FC	FC	FC	C	R	.....	.....	.....	.....	.....	.....	.....
Echinoid	R	R	R	FC	R	.....	R	.....	.....	.....	.....	FC	.....	.....	R
Sponge	FC	FC	R	R	R	R	R	.....	R	R	R	R	R	R	R
Tunicate	.....	.....	R	R	.....	FC	.....	.....	.....	.....	.....	.....	.....	.....	.....
Worm tubes	6.5	10.5	3.8	5.7	5.9	3.1	5.0	.....	tr.	tr.	0.1	1.0	tr.	.....	tr.
Pellets	.....	.....	.....	tr.	.....	.....	.....	1.0	3.6	29.5	13.0	0.9	6.8	7.5	8.0
Inorganic and Questionable Organic Constituents															
Minerals	tr.	0.6	0.8	0.4	0.3	0.5	0.4	1.5	.....	.....	.....	0.5	tr.	tr.	.....
Carbonaceous grains	R	.....	R	R	R	R	.....	FC	.....	.....	.....	.....	.....	.....	.....
Coal, cinders	.....	.....	R	R	R	.....	R	.....	.....	.....	.....	R	R	.....	R
Gypsum	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	R	R	.....	R
Magnetite	.....	R	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Mica	.....	R	.....	.....	.....	.....	R	.....	.....	.....	.....	.....	.....	.....	.....
Olivine	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	R	.....
Quartz	.....	R	.....	.....	.....	.....	FC	.....	.....	.....	.....	R	R	R	R
Volcanic ash, glass	.....	R	.....	.....	.....	.....	.....	.....	.....	.....	.....	R	.....	.....	.....
Aggregates	7.1	3.4	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
$CaCO_3$ fragments	6.5	6.8	5.1	1.5	7.9	6.7	8.9	2.7	9.0	1.0	2.1	4.5	3.8	2.4	4.8
Oolites	.....	.....	0.2	.....	1.3	.....	.....	1.1	2.7	.....	1.0	12.2	.....	.....	.....
Silt	4.5	4.8	3.5	3.2	0.5	0.6	0.4	32.0	2.4	30.2	31.1	24.1	31.1	31.5	27.6
Clay	2.8	2.7	2.0	2.3	1.3	1.0	0.4	29.3	8.0	35.3	40.1	31.4	40.2	56.4	34.8
Total	100.6	100.1	99.7	100.1	100.0	99.8	99.9	99.3	100.1	100.0	100.8	100.7	99.5	99.7	99.1

TABLE 13—*Microscopical analyses—(Continued)*
F. BAHAMA SAMPLES 182 TO 195

Organic Constituents	Sample No.								
	182	183	184a	184b	187	188	189	194	195
Algæ	10.7	2.9	23.5	22.4	36.4	34.4	38.2	26.4	28.9
Coralline	VC	FC	A	A	A	A	VC	C	VC
Halimeda	FC	R	VC	A	A	A	A	A	A
Bryozoa	.....	.....	.....	.....	.....	.....	.....	.....	.....
Coral	.....	.....	19.8	7.7	8.5	9.9	11.5	16.6	7.2
Crustacea	0.6	0.2	.....	0.4	.....	.....	0.6	.....	0.5
Decapod	R	.....	.....	.....	.....	.....	.....	.....	.....
Ostracod	R	R	.....	R	.....	.....	FC	.....	FC
Fish otoliths, teeth	.....	.....	.....	.....	.....	.....	.....	.....	.....
Foraminifera	9.0	5.0	20.8	20.8	21.0	21.6	15.8	14.9	27.3
Amphistegina	.....	.....	R	.....	.....	.....	.....	R	.....
Anomalina	.....	.....	.....	.....	.....	.....	.....	.....	.....
Archaias	C	C	VC	VC	VC	C	C	FC	A
Articulina	.....	.....	.....	R	.....	.....	R	.....	R
Asterigerina	.....	.....	FC	.....	.....	R	R	R	.....
Borelis	.....	.....	R	.....	.....	.....	.....	.....	.....
Cibicides	.....	.....	R	.....	.....	.....	.....	.....	.....
Clavulina	R	R	.....	R	FC	.....	.....	.....	R
Cymbaloporetta	.....	.....	R	.....	.....	.....	.....	.....	R
Discorbis	.....	R	R	R	.....	.....	R	.....	.....
Elphidium	R	R	.....	R	.....	.....	R	.....	R
Homotrema	.....	.....	.....	.....	.....	.....	R	.....	.....
Lagena	.....	.....	.....	FC	.....	.....	.....	.....	FC
Nonion	.....	.....	FC	R	R	C	R	R	FC
Peneroplis	.....	R	R	R	FC	.....	R	R	FC
Planorbulina	.....	.....	.....	R	.....	.....	FC	.....	R
Pyrgo	.....	.....	.....	R	R	.....	R	.....	R
Quinqueloculina	FC	C	FC	C	VC	C	C	.....	C
Rotalia	R	.....	FC	R	FC	VC	C	C	R
Sorites	.....	.....	R	FC	R	.....	R	.....	.....
Spiroloculina	.....	.....	.....	.....	.....	.....	.....	.....	FC
Verneuilina	.....	.....	FC	R	.....	.....	.....	.....	.....
Valvulina	R	R	.....	R	R	.....	.....	.....	FC
Mollusk	20.8	3.8	15.2	14.5	12.4	18.2	16.8	28.2	13.5
Gastropods	A	FC	C	VC	C	VC	VC	A	C
Pelecypods	A	FC	VC	C	C	A	VC	A	C
Spicules, fragments	tr.	tr.	4.5	6.4	2.5	5.0	4.7	4.3	2.7
Alcyonarian	.....	.....	C	A	C	C	C	C	FC
Echinoid	.....	.....	FC	R	.....	.....	R	FC	.....
Sponge	R	R	R	FC	R	FC	C	FC	FC
Tunicate	.....	.....	.....	R	.....	FC	.....	.....	R
Worm tubes	0.1	0.3	4.6	4.2	4.0	1.3	2.9	1.7	2.4
Pellets	.....	20.3	.....	.....	.....	.....	.....	.....	.....
Inorganic and Questionable Organic Constituents									
Minerals	3.6	0.6	1.0	tr.	tr.	.....	tr.	1.0	0.4
Carbonaceous grains	C	.....	FC	R	.....	.....	.....	.....	.....
Chert	.....	.....	.....	.....	.....	.....	.....	R	R
Coal, cinders	FC	R	R	.....	R	.....	.....	R	R
Gypsum	R	R	.....	.....	.....	.....	.....	R	.....
Hematite	.....	.....	R	.....	.....	.....	.....	.....	.....
Jasper	.....	.....	FC	.....	.....	.....	.....	.....	.....
Limonite	.....	FC	.....	.....	.....	.....	.....	.....	.....

TABLE 13—*Microscopical analyses*—(*Continued*)
F. BAHAMA SAMPLES 182 TO 195—(*Continued*)

Organic Constituents	Sample No.								
	182	183	184a	184b	187	188	189	193	195
Magnetite	.....	.....	R	.....	.....	.....	.....	R	.....
Olivine	R	.....	.....	.....	.....	.....	.....	.....	.....
Quartz	R	.....	R	R	.....	.....	R	FC	R
Aggregates	.....	.....	.....	5.5	1.9	.....	.....	.....	.....
$CaCO_3$ fragments	16.1	2.7	7.9	7.2	7.3	8.2	8.3	4.7	6.8
Oolites	10.7	2.3	1.6	1.8	0.9	0.7	tr.	1.7	0.2
Silt	11.6	24.9	0.2	7.1	2.9	0.2	0.3	0.1	6.3
Clay	17.6	37.5	1.0	2.5	2.1	0.3	1.1	0.7	3.6
Total	100.8	100.5	100.1	100.5	99.9	99.8	100.2	100.3	99.8

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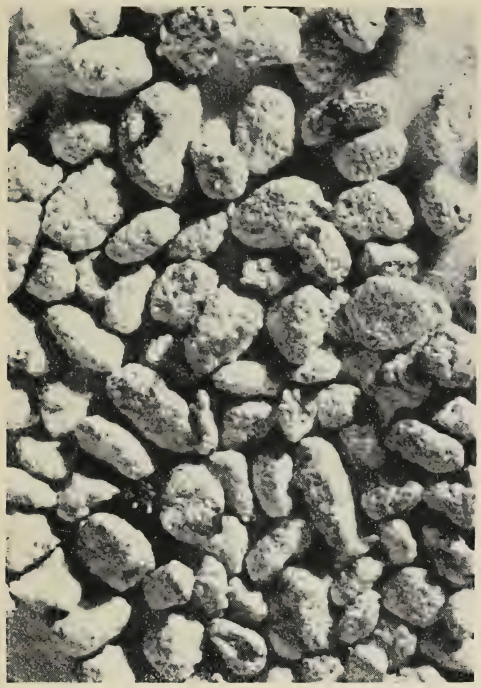

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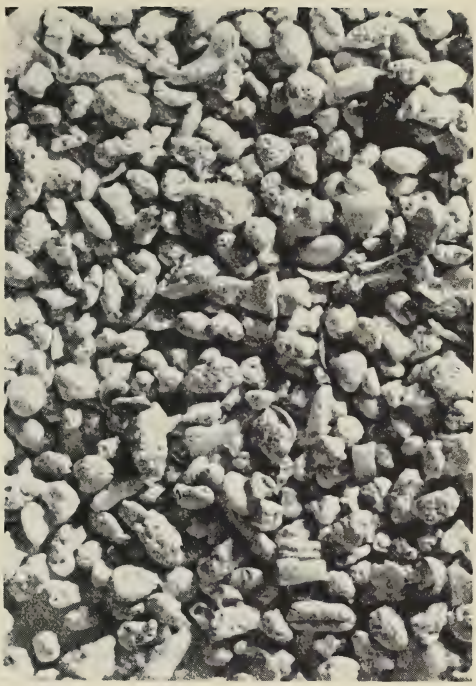
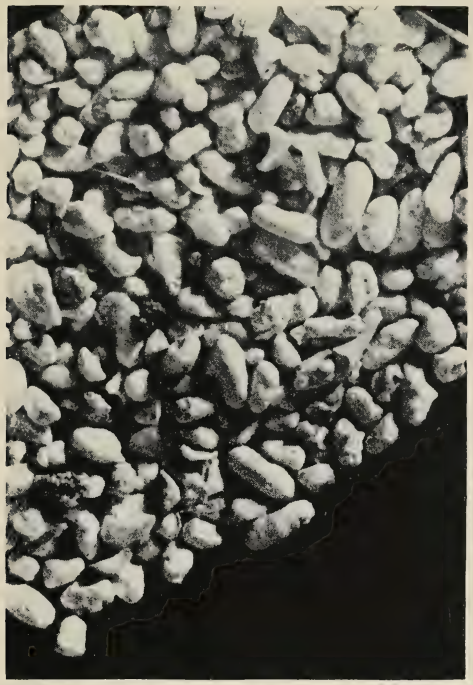


2 x 5

3 x 4

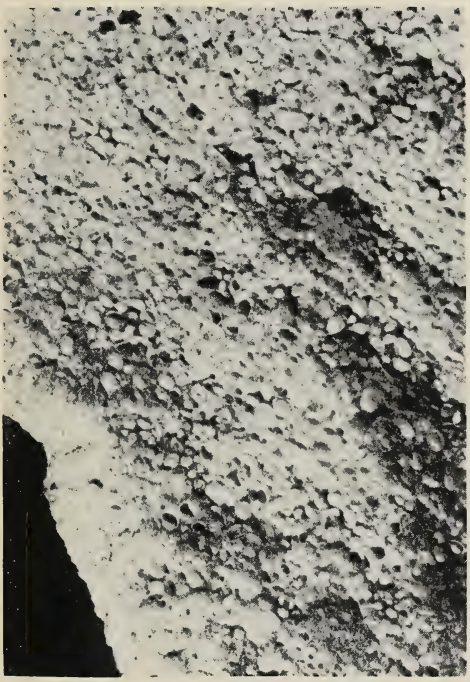
4 x 10

Fig. 1—Ellipsoidal pellets, b.s. 176b, x 10.
Fig. 2—Pellet mud showing mixture of pellets, oolites, and other calcareous débris, b.s. 176b, x 5.
Fig. 3—Typical calcareous sand, b.s. 126, x 4.
Fig. 4—Alcyonarian spicules, *Briareum* sp., from Tortugas, x 10.



1 x8

2 x8



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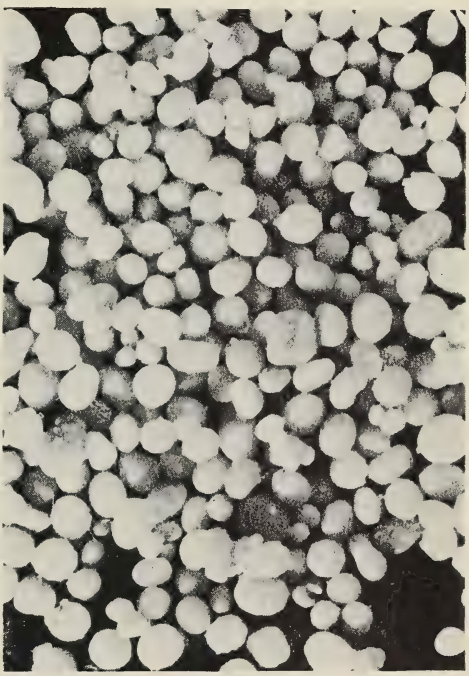
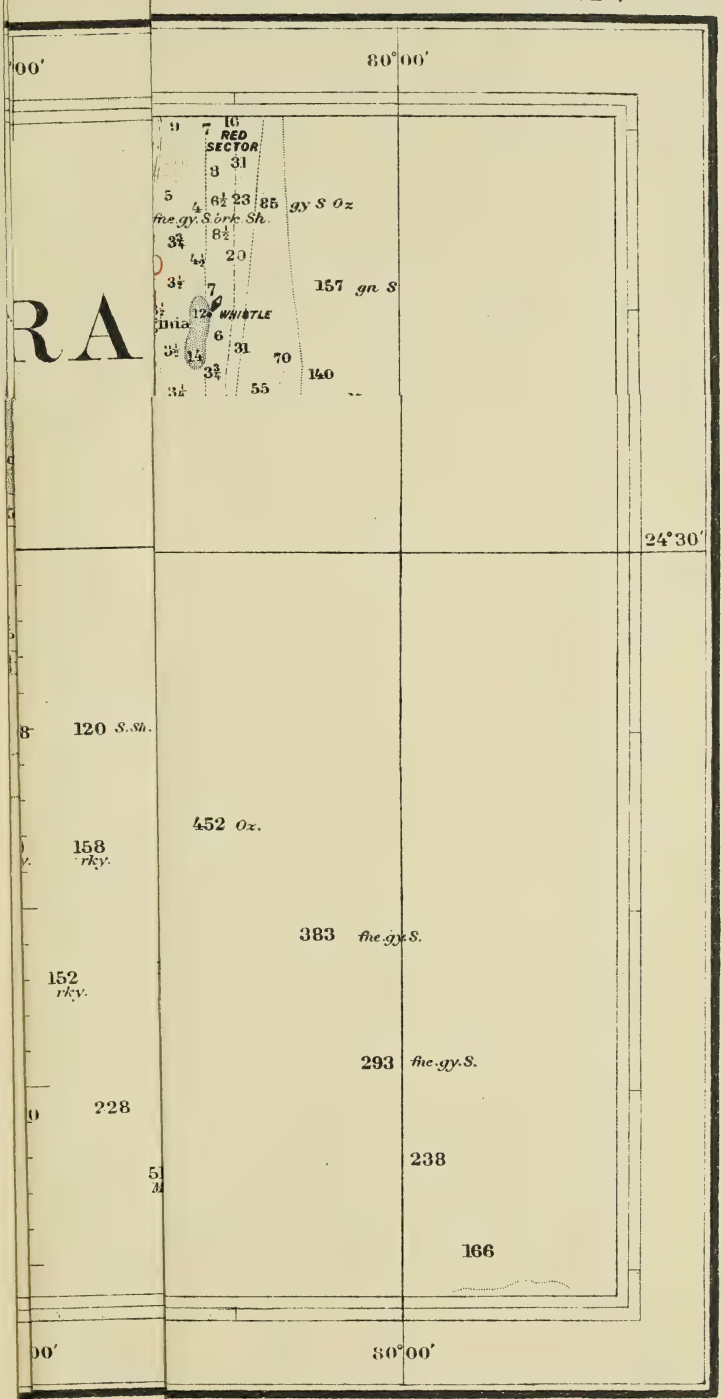


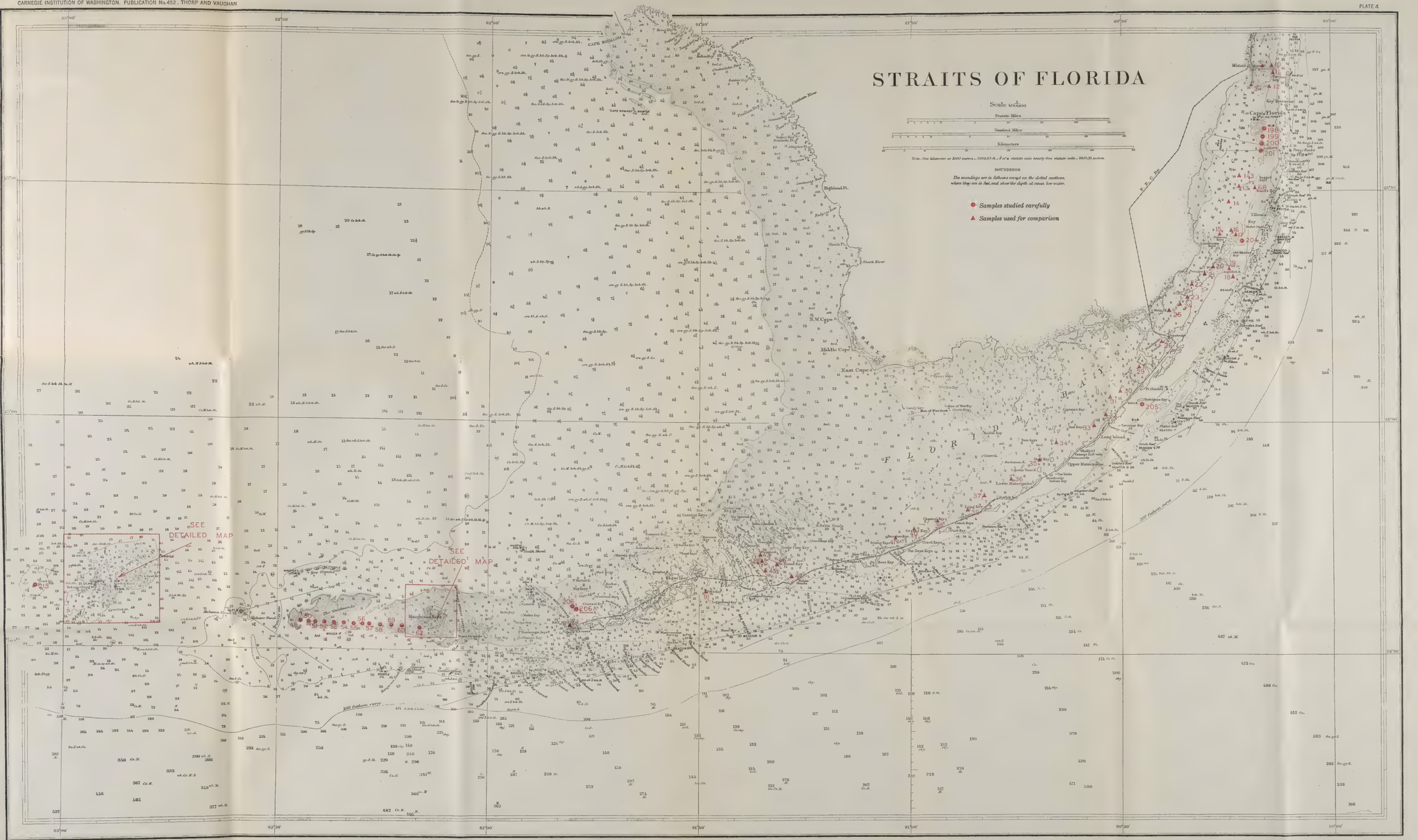
4 x6

Fig. 1—Oolites caught on 1.0-0.5 mm. screen, b.s. 181, x 8.
Fig. 2—Oolite from Abusir, Egypt, collected by Prof. F. W. Oliver, x 8.
Fig. 3—Oolite from South Andros Island, Bahamas, collected by T. W. Vaughan, 1912. x 4.
Fig. 4—Oolites from shore of Great Salt Lake, collected by T. W. Vaughan. x 6.

FIG. 1—Calcareous sand containing grains of coal, b.s. 123, Tortugas, x 10.
FIG. 2—Quartz sand, b.s. 198, x 5.

PLATE 4


80°00'
RED SECTOR
WHISTLE
24°30'
120 S.Sh.
452 Ox.
158 rky.
383 fne.gy.S.
152 rky.
293 fne.gy.S.
228
238
166
80°00'



Base from U. S. Coast and Geodetic Survey, Chart No. 15

STRAITS OF FLORIDA, SHOWING LOCATION OF BOTTOM SAMPLE STATIONS ALONG THE FLORIDA KEYS AND RELATION OF DETAILED CHARTS OF TORTUGAS AND MARQUESAS

APPENDIX 1

DIATOMS IN BOTTOM DEPOSITS FROM THE BAHAMAS AND THE FLORIDA KEYS

By Albert Mann [1]

I have thoroughly examined the twenty-four samples of calcareous sand collected by Doctor Vaughan in 1914 in the Bahamas, between Cape Florida and Key West, and at Tortugas. At the time these samples were received, I made a hasty study of them and wrote a preliminary report of their diatom contents. The following report is therefore an amplification of that one.

The amount of diatom material in the two or three ounces of calcareous sand representing each sample was in each case very small, and in some the amount was insufficient to determine the character of the diatom flora of the locality. This was particularly trying in a number of those from the Bahamas, in the neighborhood of South Bight, as the diatoms were mainly rare or actually new species; so that, as a diatomist, I had a rather painful appetite for more material.

The diatoms are, as a rule, scanty in number, and a large proportion of them are so delicate—that is so poorly silicified—as to be mere ghosts of their usual selves. They are not appreciably below normal size but below normal density in construction; and their markings are faint and hard to see. Then too, there is a relatively enormous number of cripples and misshapen individuals; as though each plant, during the period of its formation, was insufficiently supplied with the necessary amount of silica to give it external rigidity, resulting in frequent distortions. Attention was drawn to this frequency of distorted forms, as well as general ghostliness, in the diatoms I found in some material sent me from Fort Jefferson Moat, which was also a calcareous gathering.

But there are some exceptions to the foregoing which are rather startling. The silica sponge spicules, often forming a large part of the residue after the calcareous material is removed, are usually massive and well formed; and certain species of diatoms seem to be always normal in shape and sculpturing. But I think I have observed that these species are exclusively those which have a very robust and coarsely marked encasement of silica, as *Actinoptychus undulatus* (Bail.) Ralfs, *Biddulphia pulchella* Gray, *Grammatophora marina* (Lyng.) K., *Navicula lyra* E., etc. We might assume that the diminution of available silica in the sea-water would not work so disastrously in these cases as with such delicate forms as species of the genus *Mastogloia*, a genus very characteristic of all these waters. But the disturbing fact confronts us that these robust diatoms are themselves far from abundant, nor are they in greater proportion to the other kinds than in other places.

[1] Late Research Associate, Carnegie Institution of Washington.

I think that it is worthy of comment that in all the Bahama-Florida samples sent to me, diatoms are strikingly few in number, imperfectly silicified and frequently of misshapen form.

Material of this kind is very easy to prepare for diatom study. The calcareous part is removed by boiling in hydrochloric acid and washing; then the organic substances are destroyed with hydrochloric-nitric treatment. The residue, often only a fraction of 1 per cent, is made up of an occasional dinoflagellate or a radiolarian, with an abundance of sponge spicules and the diatoms. There is neither clay nor sand.

List of stations at which diatoms were collected

Sample No.		Locality	Depth Feet
Bahamas	148	South Bight	7.5
	149	South Bight	0
	174	South Bight	0.5
	177	2 miles west of the mouth of South Bight (mud 2 ft. deep)	6
	178	South Bight, west bank of entrance at west end of South Bight (mud 7 ft. deep)	2
	181	South Bight, bar at head of South Bight Canal	0
	187	North side of Scott Key, off Conch Bay	4
	188	Off Cocoanut Point and Nicollstown Light	10
	191	Off Cocoanut Point and Nicollstown Light	10
Florida	115	Marquesas Lagoon, east side—core of bottom mud	0
	121	Tortugas, Bird Key Harbor	36
	131	Tortugas Lagoon	54
	133	Tortugas	60
	198	Between Cape Florida and Soldiers Key (siliceous sand bottom)	9
	201	West end of Soldiers Key landing (some siliceous sand)	0
	204	North side of Caesars Creek bank	11
	205	Off Rodriguez Key	12
	206	2 miles north of the F.E.C.Ry. terminal station Key West	3
	206a	A little east of 206	6
	210	Tortugas	54
	212	Tortugas	48
	214	Tortugas	66

The following is a list of the diatoms found:
Bahamas; samples Nos. 148, 149, 174, 177, 178, 181, 187, 188, 191.

	Number
Actinocyclus ehrenbergii Ralfs	149
sparsus (Greg.) Ratt.	174
Actinoptychus undulatus (Bail.) Ralfs	191
Amphora angusta (Greg.) Cl.	177, 178
brasiliensis C. Zimm.	148
cymbifera Greg.	149
excisa Greg.	188
inflata Grun.	148, 187
milesiana Greg.	178
oblonga Greg.	149
obtusa Greg.	148, 149, 174, 187
ostrearia Breb.	148, 177, 178
porcellus Kitt.	177, 178, 181
proteus Greg.	148, 174, 178, 181
turgida Greg.	174, 177, 178, 181
sp. (in 177)	
Anaulus minutus Grun.	149, 187

	Number
Biddulphia pentacrinus (Wall.) V. H.	148
new species (in 191)	
Campylodiscus ecclesianus Grev.	187
hodgsonii W. S.	177
imperialis Grev.	177, 187
intermedius Grun.	149
limbatus Breb.	177, 178, 181
ralfsii W.S.	181
rotula Grun. (?)	191
samoensis Grun.	149
Climacosphenia moniligera E.	187
Cocconeis grata A.S.	188
placentula E.	188
scutellum E.	191
Coscinodiscus nitidus Greg.	187
nitidus (?) var. scintillans Per. (probably a n.s.)	188, 191
Cymatosira lorenziana Grun.	149
Epithemia gibberula K. (an introduced fresh-water form)	181
Fragilaria dubia Grun.	174, 187
Glyphodesmis eximia Grev.	149, 188
Hemidiscus cuneiformis Wall.	149
Mastogloia albifrons Br.	148
angulata Lewis	148, 149, 177, 178
apiculata W.S.	174
bahamensis Cl.	148, 149, 174, 178, 187
cocconeiformis Grun.	149
cribrosa Grun.	148, 187
cuspidata Cl.	187
elegans Lewis	148, 149, 174, 177, 178, 181, 187
erythræa Grun.	177
gilbertii A.S.	177
jelineckiana Grun.	148, 174, 187
labuensis Cl.	177
lineata Cl. & Grove	148, 177
oculiformis Br. (?)	187
ovata (Grun.) V.H.	149, 174, 177, 178
ovum-paschale (A.S.) Mann	149
rhombus (Pet.) Cl.	149
rimosa Cl.	149, 177, 178
rostatella Grun.	174, 177, 181, 187, 191
smithii Thw.	149, 181
splendida (Greg.) V.H.	191
squamosa Br.	174
new species (in 149)	
new species (in 174)	
new species (in 177)	
Melosira varians Ag.	148
Navicula advena A.S.	187
arverna Herib.	191
aspera E.	149, 187
bicuneata Grun. (var. of N. samoensis Grun. ?)	181
bomboides A.S.	148, 174
brevis Greg. var.	148
campylodiscus Grun.	149
didyma K.	174, 177, 178, 181, 187
diplosticta Grun.	149
erythræa Grun.	148, 191
formosa Greg.	148, 174, 177, 178, 181, 187
fusca var. delicatula Grun.	149, 187
irrorata Grev. var. (teste Sch. Atlas, Pl. 2, f. 19; probably n.s.)	148
littoralis Donk.	174, 187
longa W.S.	149, 177, 178, 181

	Number
lyra E.	149, 174, 188
marginata Lewis	174, 187
munda Jan.	174, 187
pandura Breb.	148
powellii Lewis	148, 174, 177, 178
samoensis Grun.	177, 181
suborbicularis Greg.	148, 149, 174, 178, 181, 187, 188
new species (in 148)	
new species (in 177)	
new species (in 188)	
new species (also in 188)	
Nitzschia granulata Grun.	188
littoralis Grun. (?)	191
plana W.S. var. (too wide a var., probably n.s.)	148, 178, 181
sigma (K.) W.S.	148, 149, 177, 178, 181, 187
sigmoidea W.S.	149
Plagiogramma atomus Grev.	149
Pleurosigma affine Grun.	148
balæricum Grun.	148
balticum W.S.	149
compactum Grun. (Rhoicosigma)	177, 187
decorum W.S. } (some unite these two)	} 187
formosum W.S. }	} 149, 177, 178, 181
oceanicum Per. (Rhoicosigma)	177
rigidum W.S.	149
Stictodesmis australis Grev.	148, 149, 177, 187
Surirella fastuosa E.	178, 181, 187
mexicana A.S.	149, 177, 187
Synedra baculus Greg.	149
fulgens W.S.	187
Tropidoneis lepidoptera (Greg.) Cl.	148, 149, 174, 177, 178, 181, 191

Marquesas, Cape Florida, Key West: Samples Nos. 115, 198, 201, 205, 206, 206a.

Actinocyclus moniliformis Ralfs	201
sparsus (Greg.) Ratt.	115, 201, 206
Actinoptychus undulatus (Bail.) Ralfs	115, 206
Amphora angusta (Greg.) Cl.	115
binodis var. interrupta Grun.	206, 206a
contracta Grun. (A. janischii A.S.)	205
cymbifera Greg.	205
obtusa Greg.	115, 198, 204, 206a
ovalis (Breb.) K.	206a
proteus Greg.	115, 198, 201, 205, 206
turgida Greg.	204, 205, 206, 206a
Anaulus minutus Grun.	201, 205
Auliscus cælatus Bail.	201
reticulatus Grev.	201
Biddulphia favus (E.) V.H.	206a
grunowii (Jan.) V.H.	201
n.s. (?) perhaps a triangular form of B. obtusa K. (in 115)	
pentacrinus (Wall.) V.H.	115, 201, 204, 205, 206, 206a
pulchella Gray	115, 201, 204, 206, 206a
rhombus (E.) W.S.	201, 205
setigera (Bail.) Mann	201, 204
tuomeyi (Bail.) Roper	115, 201, 205
sp. ? (in 201)	
Campylodiscus ecclesianus Grev.	201, 204, 206, 206a
grundleri Grun.	204
imperialis Grev.	201
intermedius Grun.	204
limbatus Breb.	204, 206a
ralfsii W.S.	204

	Number
samoensis Grun.	204, 205, 206
new species (in 204 and 205)	
Climacosphenia moniligera E.	201
Cocconeis dirupta Greg. var.	198
placentula E.	115, 205
scutellum E.	206, 206a
Coscinodiscus blandus A.S. (?)	206
nitidus var. scintillans Per. (var. too wide, probably n.s.)	115, 198, 201, 204, 206, 206a
rothii (E.) Grun.	115, 206a
Cyclotella striata (K.) Grun.	206
Cymatosira lorenziana Grun.	115, 198, 201, 204, 205, 206, 206a
Dimeregramma minus (Greg.) Ralfs	115, 201
Epithemia gibberula K. (fresh-water, introduced)	201, 204, 205, 206a
zebra K.	205
Fragilaria dubia Grun.	206a
schwartzii Grun. (Opephora)	201
Glyphodesmis eximia Grev.	201, 204, 205, 206
Hyalodiscus stelliger Bail.	205
Mastogloia amphicephala Grun.	201, 204
angulata Lewis	201, 204, 205
apiculata W.S.	201, 205
aspera Per.	201, 204, 206
bahamensis Cl.	201, 206
balkanica Br.	198
cocconeiformis Grun.	115, 198, 204, 205, 206
corsica Grun.	198
cribrosa Grun.	115, 205, 206a
cuspidata Cl.	204, 205
elegans Lewis	115, 201, 205, 206a
erythræa Grun.	204
fimbriata (Br.) Grun.	201, 206, 206a
gilbertii A.S.	206a
grunowii A.S.	205
lineata Cl. & Grove	198, 204
minuta Grev.	115, 198
ovata (Grun.) V.H.	201
rostatella Grun.	198, 204, 206a
smithii Thw.	201, 204, 206, 206a
splendida (Greg.) Mann	115, 201, 206, 206a
sp ? (in 205)	
new species (in 205)	
Melosira fausta A.S.	206, 206a
sulcata (E.) K.	115, 198, 201, 204, 205, 206, 206a
varians Ag.	198, 205
Navicula advena A.S.	205, 206
aspera E.	115, 201, 204, 205, 206
bicuneata Grun. (var. of N. samoensis Grun. ?)	205
bisulcata Lag.	206
bomboides A.S.	115
brasiliensis Grun. (type form)	115, 201, 205, 206, 206a
brasiliensis var. ? (teste Fricke, in Sch. Atlas Pl. 6, f. 32)	115
crabro E.	201, 204, 205, 206
didyma K.	204, 205, 206
digito-radiata Greg.	198, 205
forcipata Grev.	201, 206
formosa Greg.	201, 204, 205, 206a
fusca var. delicatula Grun.	204, 205, 206
ingens Mann (n.s. in Philippine Diatoms)	115
irrorata var. Mexicana Cl. (?) (probably n.s.)	205, 206a

	Number
littoralis Donk.	205, 206
longa W.S.	205, 206a
lyra E.	115, 201, 204, 205
marginata Lewis	205
maxima Greg.	115, 204, 205, 206a
munda Jan.	206
nummularia Grev. (var. of N. forcipata Grev.?)	198
pandura Breb.	205
plicata Donk.	205
robusta Grun. var.	205
samoensis Grun.	206
serrulata Grun.	201
suborbicularis Greg.	201, 205, 206a
tripartita Mann (n. s. in Philippine Diatoms)	198
weissflogii A.S.	115
zostereti Grun.	198
sp. (in 198)	
new species (in 198)	
Nitzschia dubia Grun.	204, 205, 206
marginulata var. didyma Grun.	206a
panduriformis Greg.	115, 201, 204, 205, 206a
plana W.S. (?)	115, 201, 204, 205, 206a
romana Grun.	201
sigma (K.) W.S.	115, 204, 206, 206a
sigma var. rigida Grun.	204
valida Cl. & Grun.	204
Plagiogramma gregorianum Grev.	205
nankoorense var. biconstricta Grove & Grun. (P. kinkeri A.S.)	204, 206a
pulchellum Grev.	201
Pleurosigma affine Grun.	204
balticum W.S.	206a
decorum W.S.	205
formosum W.S.	115, 201, 204, 205, 206a
lineare Grun.	204
rigidum W.S.	201
Rhabdonema mirificum Grev.	198
Sceptroneis marina Grun. (Opephora)	115
Stictodesmis australis Grev.	204, 205
Surirella fastuosa E.	201, 204, 205, 206, 206a
mexicana A.S.	205
new species (in 206)	
Synedra baculus Greg.	206
crystallina (Ag.) K.	115, 205
cuneata Grun.	204
superba W.S.	198, 201, 204, 206, 206a
Trigonium sculptum (Shad.) Cl.	115, 201, 204, 205
Tropidoneis lepidoptera (Greg.) Cl.	115, 198, 204, 205

Tortugas: Samples Nos. 121, 131, 133, 210, 212, 214

Achnanthes longipes Ag.	131
Actinocyclus moniliformis Ralfs	214
sparsus (Greg.) Ratt.	121, 131, 210
Actinoptychus undulatus (Bail.) Ralfs	121, 131, 133, 210, 212, 214
Amphora binodis var. interrupta Grun.	131, 210, 212, 214
contracta Grun. (A. janischii A.S.)	214
crassa Greg.	214
inflata Grun.	212
lunula Cl.	214
mexicana A.S.	210
obtusa Greg.	131, 210, 212
proteus Greg.	131, 210, 214

	Number
schmidtii Grun.	212
turgida Greg.	210, 212
Anaulus minutus Grun.	121
Biddulphia favus (E.) V.H.	121, 131 and quadrate form 121
pentacrinus (Wall.) V.H.	121, 133, 210, 214
pulchella Gray	212
rhombus (E.) W.S.	121, 133, 212
tuomeyi (Bail.) Roper	131, 133, 210, 212, 214
turgida (E.) W.S.	214
Campylodiscus imperialis Grev.	121
rotula Grun.	131
Chaetoceros coarctatus Laud.	121
varians (Laud.) V.H. (Bacteriastrum)	214
Cocceneis distans Greg.	131, 133
placentula E.	131
scutellum E.	131
Coscinodiscus excentricus E.	131
nitidus var. scintillans Per. (var. too wide; probably n.s.)	121, 131, 212, 214
nodulifer A.S.	121, 131, 212
rothii (E.) Grun.	121, 131, 133, 210, 212, 214
subtilis E.	212, 214
woodwardii Eul.	214
Cyclotella striata (K.) Grun.	131, 212
Cymatosira lorenziana Grun.	121, 131, 133, 210, 212
Dimeregramma minus (Greg.) Ralfs	121, 131, 212
new species (in 210 and 214)	
Epithemia gibberula K.	210, 212
Fragilaria elliptica Schum.	212
Glyphodesmis eximia Grev.	133, 210, 214
Hyalodiscus stelliger Bail.	131, 212, 214
subtilis Bail.	133
Isthmia enervis E.	131
Mastogloia angulata Lewis	212
apiculata W.S.	210, 212
bahamensis Cl.	212
balkanica Br.	212
braunii Grun.	212
cocconeiformis Grun.	131
elegans Lewis	210, 212
jelineckiana Grun.	121, 133
ovata (Grun.) V.H.	121
rostatella Grun.	212
undulata Grun.	121
new species (in 210 and 212)	
Melosira fausta A.S.	214
sulcata (E.) K.	121, 131, 133, 210, 212, 214
Navicula approximata Grev.	212
aspera E.	121, 210
bombus E.	133, 210, 212
brasiliensis Grun. (type)	121, 131, 210, 214
brasiliensis var. ? (teste Fricke, in Sch. Atlas, Pl. 6, f. 32)	212
clavata Greg.	131
crabro E.	121, 131, 133
didyma K.	214
eudoxia A.S.	133
forcipata Grev.	131, 133
formosa Greg.	214
gemmulata Grun.	131
hennedyi W.S. and var.	131, 133
ingens Mann (n.s. in Philippine Diatoms)	121
irrorata var. mexicana Cl. (?) (probably n.s.)	121, 131, 210
littoralis Donk.	212

	Number
longa W.S.	210, 212, 214
lyra E. (type)	121, 210, 212, 214
lyra var. elliptica A.S.	131, 133
lyra var. recta Grev.	131, 133, 210
marginata Lewis	131, 212, 214
maxima Greg.	210, 212
munda Jan.	210
muscæformis Grun.	212
pandura Breb.	131, 212
spectabilis var. emarginata Cl.	210
subcincta A.S.	210
suborbicularis Greg.	131, 133, 210, 212, 214
thaitiana (Cast.) Mann (Stauroneis)	210
weissflogii A.S.	121, 133, 210, 212, 214
sp. (in 133)	
new species (in 212)	
Nitzschia bombiformis Grun.	133
marginulata var. didyma Grun.	121, 131, 214
panduriformis Greg.	121, 131, 133, 210, 212
plana W.S. (?)	121, 131, 133, 214
sigma (K.) W.S.	121, 210, 212, 214
Pleurosigma balticum W.S.	214
latum Grun.	121, 131, 212, 214
rigidum W.S.	212
Rhaphoneis amphiceros var. tetragona Grun.	212
belgica Grun.	133
Sceptroneis marina Grun.	121
Surirella fastuosa E.	121, 131, 133, 212, 214
mexicana A.S.	131, 210, 214
reniformis Grun.	131, 212
Synedra crystallina (Ag.) K.	214
superba W.S.	131
Trigonium sculptum (Shad.) Cl.	121, 131, 212
Tropidoneis lepidoptera (Greg.) Cl.	212

Species of the genus *Mastogloia* are very abundant in these Florida samples, and I have found them to be so in all collections from Florida waters. In other parts of the world they are relatively much less abundant. They are as a class delicate forms and, as above stated, they suffer greatly from the lack of silica in all the gatherings here included.

APPENDIX 2

CURRENT MEASUREMENTS ALONG THE FLORIDA CORAL REEF TRACT

By Thomas Wayland Vaughan [1]

During June and July 1914, while studying the phenomena associated with the Florida Coral-Reef Tract as a guest of the Tortugas Laboratory of the Carnegie Institution of Washington, I initiated a series of current measurements by using Ekman current meters provided by Dr. Alfred G. Mayor, then Director of the Tortugas Laboratory. At first I personally made the measurements, but they were soon taken over by Mr. George Tracey, the skipper, and Mr. John Mills, the engineer, of the yacht *Anton Dohrn*. The measurements during October and November 1914, at stations 15 to 19, inclusive, were made by Mr. Mills after the end of the season's work at Tortugas. My thanks are due Doctor Mayor and Messrs. Tracey and Mills.

The purpose of the measurements was to procure information on the possible effects of the currents on the distribution of sediments and the configuration of the keys. Where the water is shallow between the keys, the bottom is swept clean of sediment by the strong oscillating tidal currents;

Table 1—*Currents at stations Nos. 1, 2 and 3.*
(*For the positions of stations, see current records on following pages.*)

Date	Station No.	Time	Velocity cm. per second	Direction from which current flows	Tide
1914		*h. m. h. m.*			
June 4	1	8 30–8 45 a. m.	10.82	S. 50° W.	Ebb
	2	1 23–1 53 p. m.	9.74	N. 60° E.	Flow
	3	2 15–2 45	15.98	N. 60° E.	Flow
	3	5 30–6 00	12.98	N. 30° E.	Ebb
June 5	3	6 40–6 55 a. m.	33.86	S. 50° E.	Ebb

but where the water is deeper, as in Tortugas Lagoon, relatively fine-grained sediments accumulate on bottoms above which at the surface the currents may be rapid.

It was also desired to test the actuality of the Florida Counter Current. The measurements at the first three stations were not conducted at any one station over the complete tidal swing, but the stations were in Hawk Channel and were rather close together. Combining the records at the three stations gives the result as is shown in table 1.

[1] Director, Scripps Institution of Oceanography, University of California, La Jolla, California.

This table suggests more movement from the east toward the west than in the opposite direction. At station 4, off Rodriguez Key, Hawk Channel, the current rosette shows more motion toward the west than toward the east. South of the Tortugas, at stations 7 and 13, a non-tidal current toward the west is clearly indicated.

Although the data here presented are inadequate for positive conclusions regarding the Counter Current, they are in accord with the indications on the Pilot Charts published by the U. S. Hydrographic Office.

Mr. Frank J. Haight, associate mathematician of the U. S. Coast and Geodetic Survey, has kindly prepared the discussion of the results which follow the records of observations contained in table 2.

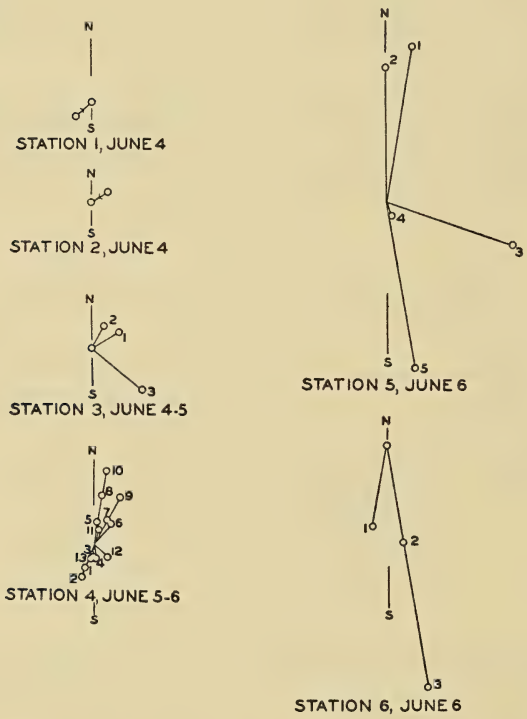


Fig. 11—Current rosettes for stations 1, 2, 3, 4, 5, and 6. Currents are represented as flowing toward station. Sequence of readings, which were hourly, is shown by numbers beginning with 1. Velocity is indicated by the length of line, scale: 1 mm.=4 cm. per second. 1 cm. per second=0.0194 knots per hour.

TABLE 2—*Current records, Florida reef tract.*

Date, June 1914	Time of day	Current			Wind	Tide
		Velocity		Direction		
		Cm. per second	Knots per hour			
	Station No. 1, off Soldier's Key, red buoy No. 2, Hawk Channel Lat. 25° 33.3′, Long. 80° 7.5′ (see U. S. C. & G. S. chart 166)					
4	*h. m. h. m.* 8 30–8 45 a. m.	10.82	0.211	S. 50° W.	S.E. light	Ebbing
	Station No. 2, Caesar's Creek Point, Hawk Channel Lat. 25° 23.3′, Long. 80° 11.3′ (see U. S. C. & G. S. chart 166)					
4	*h. m. h. m.* 1 23–1 53 p. m.	9.74	0.180	N. 60° E.	E. light	Flowing
	Station No. 3, off mouth of Caesar's Creek, north side Caesar's Creek Bank (see U. S. C. & G. S. chart 166)					
	h. m. h. m.					
4	2 15–2 45 p. m.	15.98	0.310	N. 60° E.	E. light	Flowing
4	5 30–6 00	12.98	.252	N. 30° E.	E. light	Ebbing
5	6 40–6 55 a. m.	33.86	.657	S. 50° E.	S.E. light	Ebbing
	Station No. 4, off Rodriguez Key, Hawk Channel, depth 12 ft. Lat. 25° 02.2′ N., Long. 80° 26.2′ W. (see U. S. C. & G. S. chart 167)					
	h. m. h. m.					
5	10 55–11 10 a. m.	13.10	0.254	S. 20° W.	S.E. light	Ebbing
5	2 15–2 30 p. m.	4.82	.094	S. 20° W.	S.E. almost calm	Nearly slack
5	2 40–2 55	6.28	.122	S.	S.E. almost calm	Flowing
5	3 15–3 30	6.98	.135	S. 10° E.	S.E. almost calm	Flowing
5	4 00–4 15	11.42	.222	N. 10° E.	E. light	Flowing
5	5 10–5 15	14.18	.275	N. 40° E.	E. light	Flowing
5	6 00–6 15	14.16	.275	N. 30° E.	E. light	Flowing
5	7 00–7 15	14.18	.275	N. 10° E.	E. light	Flowing
5	8 00–8 15	14.16	.275	N. 30° E.	E. light	Flowing
5	9 00–9 15	13.12	.254	N. 10° E.	E. light	Flowing
5	10 00–10 15	8.04	.156	N. 20° E.	E. light	Flowing
5	11 00–11 15	9.86	.191	S. 40° E.	E. light	Ebbing
6	6 10–6 15 a. m.	7.10	.138	S. 10° W.	S.E. light	Ebbing

TABLE 2—*Current records, Florida reef tract—(Continued)*

Date, June 1914	Time of day	Current: Velocity: Cm. per second	Current: Velocity: Knots per hour	Current: Direction	Wind	Tide
	Station No. 5, North Side of Viaduct at Knights Key Lat. 24° 42.5′ N., Long. 81° 08.2′ W. (see U. S. C. & G. S. chart 168)					
	h. m. h. m.					
6	1 10–1 25 p. m.	82.46	1.600	N. 10° E.	E. light	Ebbing
6	2 00–2 15	69.98	1.357	N.	E. light	Ebbing
6	3 00–3 15	58.10	1.127	S. 70° E.	E. light	Ebbing
6	4 00–4 15	7.94	.154	S. 20° E.	E. light	Flowing
6	5 00–5 15	87.86	1.705	S. 10° E.	S.E. light	Flowing
	Station No. 6, South Side of Viaduct at Knights Key, depth 3 fathoms Lat. 24° 42.2′ N., Long. 81° 08.2′ W. (see U. S. C. & G. S. chart 168)					
	h. m. h. m.					
6	6 00–6 15 p. m.	42.98	0.834	S. 10° W.	S.E. light	Flowing
6	7 00–7 15	66.26	1.285	S. 10° E.	S.E. light	Flowing
6	8 00–8 15	61.18	1.187	S. 10° E.	S.E. light	Flowing
7	5 45–6 00 a. m.	66.62	1.292	S.	E. light (est. 8 mi. per hr.)	Flowing
7	7 00–7 15	71.73	1.391	S.	E. light	Flowing
7	8 00–8 15	61.05	1.184	S. 10° E.	E. light	Flowing
7	9 00–9 15	20.95	.407	S.	E. strong (est. 12 mi. per hr.)	
7	10 00–10 15	59.06	1.146	N.	E. strong	Ebbing
7	11 00–11 15	26.54	0.515	N. 20° W.	E. strong	Ebbing
7	12 01–12 16 p. m.	90.02	1.746	N. 10° W.	E. moderate	Ebbing
7	1 00–1 15	100.34	1.947	N. 20° W.	E. moderate	Ebbing
7	2 00–2 15	122.18	2.361	N. 20° W.	E. moderate	Ebbing
7	3 00–3 15	42.86	0.831	N.	E. moderate	Ebbing
7	4 00–4 15	20.06	.389	N. 20° E.	E. moderate	Ebbing
7	5 00–5 15	63.74	1.237	S. 10° E.	E. moderate	Flowing
7	6 00–6 15	67.22	1.302	S. 20° E.	E. moderate	Flowing
	Station No. 7, south side of Loggerhead buoy, Tortugas, depth 7 fathoms, meter down 3 fathoms Lat. 24° 36.6′ N., Long. 82° 57.5′ N. (see accompanying chart)					
	h. m. h. m.					
15	7 00– 7 15 a. m.	13.93	0.270	N. 20° E.	Calm	Ebbing
15	8 00– 8 15	9.86	.191	N. 60° E.	Calm	Ebbing
15	9 00– 9 15	8.65	.168	E.	N. very light	Flowing
15	10 00–10 15	16.79	.325	S. 60° E.	N. very light	Flowing
15	11 00–11 15	24.60	.477	S. 50° E.	N. very light	Flowing
15	12 00–12 15 p. m.	26.47	.514	S. 50° E.	N. very light	Flowing
15	1 00– 1 15	27.68	.537	S. 30° E.	Calm	Flowing
15	2 00– 2 15	22.18	.430	S. 30° E.	Calm	Flowing
15	3 00– 3 15	1.50	.029	S. 20° E.	Calm	Flowing
15	4 00– 4 15	10.30	.200	N. 30° W.	Calm	Ebbing

TABLE 2—*Current records, Florida reef tract—(Continued)*

Station No. 7, south side of Loggerhead buoy, Tortugas, depth 7 fathoms, meter down 3 fathoms —*Continued*
Lat. 24° 36.6′ N., Long. 82° 57.5′ N. (see accompanying chart)

Date, June 1914	Time of day	Current			Wind	Tide
		Velocity		Direction		
		Cm. per second	Knots per hour			
	h. m. h. m.					
15	5 00– 5 15 p. m.	4.03	0.078	N. 40° W.	Calm	Ebbing
15	6 00– 6 15	19.32	.375	N. 30° W.	Calm	Ebbing
15	7 00– 7 15	27.90	.541	N. 10° W.	Calm	Ebbing
21	6 30– 6 45 a. m.	9.97	.193	E.	S. E. light	High at 6:56 a. m. Flowing
21	7 30– 7 45	33.40	.648	E.	S.E. light	Ebbing
21	8 30– 8 45	2.93	.057	E.	S.E. light	Ebbing
21	9 30– 9 45	16.90	.328	N. 60° E.	S.E. light	Ebbing
21	10 30–10 45	15.36	.298	N. 60° E.	S.E. light	Ebbing
21	11 30–11 45	12.94	.251	E.	S.E. light	Ebbing
21	12 30–12 45 p. m.	38.90	.755	S. 60° E.	S.E. light	Ebbing
21	1 30– 1 45	26.47	.514	S. 50° E.	S.E. light	Ebbing
21	2 30– 2 45	22.51	.437	S. 50° E.	S.E. light	Ebbing
21	3 30– 3 45	26.14	.507	S. 50° E.	S.E. light	Low at 3:10, Flowing
21	4 30– 4 45	66.07	1.282	S. 40° E.	S.E. light	Flowing
21	5 30– 5 45	32.52	.631	S. 20° E.	S.E. light	Flowing
21	6 30– 6 45	29.11	.565	S. 30° E.	S.E. light	Flowing
Station No. 8, off White Shoal, Tortugas, between buoys C3 and N4, Southwest Channel, depth 11 fathoms Lat. 24° 37.7′ N., Long. 82° 54′ W. (see accompanying chart)						
	h. m. h. m.					
15	7 00– 7 15 a. m.	43.70	0.848	N. 30° W.	N.E. calm	Ebbing
15	8 00– 8 15	45.38	.880	N. 50° W.	N.E. calm	Ebbing
15	9 00– 9 15	31.12	.604	N. 60° E.	N.E. calm	Ebbing
15	10 00–10 15	17.54	.240	N. 70° E.	N.E. calm	Ebbing
15	11 00–11 15	3.74	.073	S. 10° W.	N.E. calm	Flowing
15	12 00–12 15 p. m.	20.78	.393	S. 20° W.	N. calm	Flowing
15	1 00– 1 15	34.82	.676	S. 20° W.	N. light	Flowing
15	2 00– 2 15	45.14	.876	S.	N. light	Flowing
15	3 00– 3 15	33.62	.652	S. 30° W.	N. calm	Flowing
15	4 00– 4 15	18.82	.365	S. 60° W.	N. calm	Flowing
15	5 00– 5 15	7.46	.145	S. 80° W.	No wind calm	Ebbing
15	6 00– 6 15	14.30	.277	N. 80° W.	No wind calm	Ebbing
15	7 00– 7 15	15.26	.296	N. 60° W.	No wind calm	Ebbing
Station No. 9, off east side Loggerhead Key, Tortugas, depth 5 fathoms; meter down 3 fathoms Lat. 24° 38.1′ N., Long. 82° 54.8′ W. (see accompanying chart)						
	h. m. h. m.					
22	6 00– 6 15 a. m.	12.83	0.249	S. 20° W.	S.E. light	Flowing
22	7 00– 7 15	31.64	.614	S. 10° W.	S.E. light	Flowing

TABLE 2—*Current records, Florida reef tract—(Continued)*

Station No. 9, off east side Loggerhead Key, Tortugas, depth 5 fathoms; meter down 3 fathoms (*Continued*)
Lat. 24° 38.1′ N., Long. 82° 54.8′ W. (see accompanying chart)

Date, June 1914	Time of day	Current			Wind	Tide
		Velocity		Direction		
		Cm. per second	Knots per hour			
	h. m. h. m.					
22	8 00– 8 15 a. m.	9.20	0.018	E.	S.E. light	Ebbing
22	9 00– 9 15	26.47	.514	N. 20° E.	S.E. light	Ebbing
22	10 00–10 15	27.90	.541	N. 20° E.	S.E. moderate	Ebbing
22	11 00–11 15	29.00	.563	N. 40° E.	S.E. moderate	Ebbing
22	12 00–12 15 p. m.	48.80	.947	N. 40° E.	S.E. light	Ebbing
22	1 00– 1 15	40.11	.778	N. 20° E.	S.E. light	Ebbing
22	2 00– 2 15	40.33	.782	N. 30° E.	S.E. light	Ebbing
22	3 00– 3 15	15.91	.309	S. 30° W.	S.E. light	Flowing
22	4 00– 4 15	42.42	.823	S. 30° W.	S.E. light	Flowing
22	5 00– 5 15	69.59	1.350	S. 30° E.	S.E. light	Flowing
22	6 00– 6 15	98.65	1.914	S. 30° E.	S.E. light	Flowing
22	7 00– 7 15	104.35	2.024	S. 40° W.	S.E. light	Flowing
22	8 00– 8 15	34.72	.674	S. 40° W.	S.E. light	Flowing
22	9 00– 9 15	31.53	.612	S. 40° W.	S.E. light	Flowing
23	5 00– 5 15 a. m.	22.62	.439	N. 20° E.	E.N.E. light	Flowing
23	6 00– 6 15	7.66	.149	S.	E.N.E. light	Flowing
23	7 00– 7 15	9.20	.178	S.	E.N.E. light	Flowing
23	8 00– 8 15	8.30	.161	S.	E.N.E. light	Flowing
23	9 00– 9 15	13.38	.260	N. 20° E.	E.N.E. light	Ebbing
23	10 00–10 15	30.54	.592	N. 20° E.	E. light	Ebbing
23	11 00–11 15	48.80	.947	N. 30° E.	E. light	Ebbing
23	12 00–12 15 p. m.	53.64	1.041	N. 40° E.	E. light	Ebbing
23	1 00– 1 15	52.10	1.011	N. 40° E.	E. light	Ebbing
23	2 00– 2 15	38.46	.746	N. 20° E.	E. light	Ebbing
23	3 00– 3 15	30.21	.586	N.	E. light	Ebbing
23	4 00– 4 15	16.57	.321	S. 30° W.	E. moderate	Flowing
23	5 00– 5 15	61.56	1.194	S. 30° W.	E. moderate	Flowing
23	6 00– 6 15	94.23	1.829	S. 10° E.	E. moderate	Flowing
24	6 00– 6 15 a. m.	5.02	.097	S.	E. light	Flowing
24	7 00– 7 15	35.71	.693	E.	E. light	Flowing
24	8 00– 8 15	13.27	.257	S. 10° E.	E. light	Flowing
24	9 00– 9 15	3.26	.063	S. 10° E.	E. light	Flowing
24	10 00–10 15	21.85	.424	N. 30° E.	E. light	Ebbing
24	11 00–11 15	46.93	.902	N. 30° E.	E. light	Ebbing
24	12 00–12 15 p. m.	59.25	1.159	N. 20° E.	E. light	Ebbing
24	1 00– 1 15	75.09	1.457	N. 30° E.	E.N.E. light	Ebbing
24	2 00– 2 15	32.52	.631	N. 10° E.	E.N.E. light	Ebbing
24	3 00– 3 15	31.86	.618	N. 20° E.	E.N.E. light	Ebbing
24	4 00– 4 15	9.42	.183	N.	E.N.E. light	Ebbing
24	5 00– 5 15	34.72	.674	S. 30° W.	E.N.E. light	Flowing
24	6 00– 6 15	63.65	1.235	N. 80° W.	N. light	Flowing
24	7 00– 7 15	69.37	1.346	S. 20° W.	N. light	Flowing
24	8 00– 8 15	72.23	1.401	S. 30° W.	N. light	Flowing
24	9 00– 9 15	58.04	1.126	N. 60° E.	N. light	Flowing
25	6 00– 6 15 a. m.	10.19	.106	N. 30° E.	E.N.E. light	Flowing
25	7 00– 7 15	3.92	.076	N.	E.N.E. light	Flowing
25	8 00– 8 15	4.69	.091	S.	E.N.E. light	Ebbing
25	9 00– 9 15	4.58	.089	N. 60° E.	E. light	Ebbing

TABLE 2—*Current records, Florida reef tract—(Continued)*

Date, June 1914	Time of day	Current			Wind	Tide
		Velocity		Direction		
		Cm. per second	Knots per hour			
	Station No. 9, off east side Loggerhead Key, Tortugas, depth 5 fathoms; meter down 3 fathoms (*Continued*) Lat. 24° 38.1′ N., Long. 82° 54.8′ W. (see accompanying chart)					
	h. m. h. m.					
25	10 00–10 15 a. m.	14.48	0.281	N. 10° E.	E. light	Ebbing
25	11 00–11 15	35.93	.697	N. 10° E.	E. light	Ebbing
25	12 00–12 15 p. m.	47.59	.923	N. 30° E.	E. light	Ebbing
25	1 00– 1 15	53.42	1.036	N. 50° E.	E. light	Ebbing
25	2 00– 2 15	55.40	1.075	N. 50° E.	E. light	Ebbing
25	3 00– 3 15	55.52	1.077	N. 30° E.	E. light	Ebbing
25	4 00– 4 15	26.58	.516	N. 20° E.	N.E. light	Ebbing
25	5 00– 5 15	11.40	.121	S. 10° W.	N.E. light	Flowing
25	6 00– 6 15	42.20	.819	S. 10° W.	N.N.E. light	Flowing
25	7 00– 7 15	63.65	1.235	S. 30° W.	N.N.E. light	Flowing
25	8 00– 8 15	86.75	1.683	S. 30° W.	N.N.E. light	Flowing
25	9 00– 9 15	70.36	1.365	N. 10° W.	N.N.E. light	Flowing
	Station No. 10, off west side of Loggerhead Key, Tortugas, depth 10 feet; meter down 4 feet (1,500 feet west of Lab. Landing) Lat. 24° 38.2′ N., Long. 82° 55.4′ W. (see accompanying chart)					
	h. m. h. m.					
23	6 00– 6 15 a. m.	7.34	0.142	S. 40° E.	E.N.E. light	Flowing
23	7 00– 7 15	8.54	.166	S. 10° W.	E.N.E. light	Flowing
23	8 00– 8 15	12.50	.243	N. 10° E.	E.N.E. light	Flowing
23	9 00– 9 15	15.02	.291	N. 20° E.	E. light	Ebbing
23	10 00–10 15	16.70	.324	S. 10° E.	E. light	Ebbing
23	11 00–11 15	14.78	.287	S. 60° E.	E. light	Ebbing
23	12 00–12 15 p. m.	5.18	.100	E.	E. light	Ebbing
23	1 00– 1 15	3.86	.075	S. 10° E.	E. light	Ebbing
23	2 00– 2 15	2.18	.042	S. 10° W.	E. light	Ebbing
23	3 00– 3 15	7.12	.138	S. 10° E.	E. moderate	Flowing
23	4 00– 4 15	3.26	.063	S. 70° E.	E. moderate	Flowing
23	5 00– 5 15	24.26	.471	S. 70° E.	E. moderate	Flowing
23	6 00– 6 15	1.70	.003	S. 70° E.	E. moderate	Flowing
	Station No. 11, Southeast Channel, Tortugas, between Bush Key and buoy No. 2, depth 13 fathoms Lat. 24° 37.4′ N., Long. 82° 50.7′ W. (see accompanying chart)					
	h. m. h. m.					
24	6 15– 6 30 a. m.	15.38	0.298	S. 30° E.	E. light	Flowing
24	7 00– 7 15	13.22	.256	S.	E. light	Flowing
24	8 00– 8 15	10.58	.205	S. 10° W.	E. light	Flowing
24	9 00– 9 15	9.14	.177	N. 40° W.	E. light	Ebbing
24	10 00–10 15	33.14	.643	N. 20° W.	E. light	Ebbing
24	11 00–11 15	36.38	.706	W.	E. light	Ebbing
24	12 00–12 15 p. m.	45.98	.892	N. 40° W.	E. light	Ebbing

TABLE 2—*Current records, Florida reef tract—(Continued)*

Date, June 1914	Time of day	Current: Velocity: Cm. per second	Current: Velocity: Knots per hour	Current: Direction	Wind	Tide
	Station No. 11, Southeast Channel, Tortugas, between Bush Key and buoy No. 2, depth 13 fathoms —(*Continued*).					
	Lat. 24° 37.4′ N., Long. 82° 50.7′ W. (see accompanying chart)					
	h. m. h. m.					
24	1 00– 1 15 p. m.	34.46	0.669	N. 30° W.	E. light	Ebbing
24	2 00– 2 15	18.26	.354	N. 20° W.	E. light	Ebbing
24	3 00– 3 15	13.46	.261	S. 30° E.	E. light	Flowing
24	4 00– 4 15	30.62	.594	S. 10° E.	E. light	Flowing
24	5 00– 5 15	51.50	.999	S. 10° E.	E. light	Flowing
24	6 00– 6 15	56.18	1.090	S. 10° E.	E. light	Flowing
	Station No. 12, Northwest Channel, Tortugas, buoy N., depth 8 fathoms					
	Lat. 24° 41.9′ N., Long. 82° 52.8′ W. (see accompanying chart)					
	h. m. h. m.					
25	6 07– 6 22 a. m.	15.50	0.301	S. 70° E.	E.N.E. very light	Flowing
25	7 00– 7 15	12.74	.247	N.	E.N.E. very light	Ebbing
25	8 00– 8 15	8.50	.165	N. 20° W.	E.N.E. light	Ebbing
25	9 00– 9 15	10.02	.194	N. 80° W.	E. very light	Ebbing
25	10 00–10 15	7.34	.143	N. 30° W.	E. very light	Ebbing
25	11 00–11 15	22.58	.438	N. 30° W.	E. very light	Ebbing
25	12 00–12 15 p. m.	38.54	.748	N. 10° W.	E. light	Ebbing
25	1 00– 1 15	42.62	.827	N.	E. light	Ebbing
25	2 00– 2 15	36.74	.713	N. 10° W.	E. calm	Ebbing
25	3 00– 3 15	26.54	.515	N.	E. calm	Ebbing
25	4 00– 4 15	11.78	.229	N. 10° E.	N.E. calm	Ebbing (?)
25	5 00– 5 15	7.58	.147	N. 80° E.	N.E. calm	Flowing
25	6 00– 6 15	32.18	.624	S. 20° E.	N.N.E. calm	Flowing
	Station No. 13, Tortugas buoy, Tortugas					
	Lat. 24° 35.1′ N., Long. 82° 54.1′ W. (see accompanying chart)					
	h. m. h. m.					
19	6 00– 6 15 a. m.	14.04	0.272	E.	S.E. light	Ebbing
19	7 00– 7 15	11.84	.230	E.	S.E. light	Ebbing
19	8 00– 8 15	19.76	.383	E.	S.E. light	Ebbing
19	9 00– 9 15	38.68	.750	E.	S.E. light	Ebbing
19	10 00–10 15	25.92	.503	E.	S.E. light	Ebbing
19	11 00–11 15	22.18	.430	E.	S.E. light	Ebbing
19	12 00–12 15 p. m.	23.50	.456	E.	S.E. light	Ebbing
19	1 00– 1 15	29.33	.568	E.	S.E. light	Ebbing
19	2 00– 2 15	32.85	.637	S. 40° E.	S.E. light	Ebbing
19	3 00– 3 15	38.02	.738	E.	S.E. light	Ebbing
19	4 00– 4 15	33.40	.648	E.	S.E. light	Ebbing
19	5 00– 5 15	24.16	.469	S. 40° E.	S.E. light	Ebbing

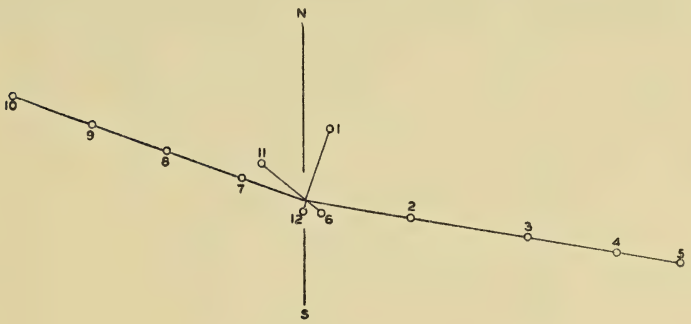
TABLE 2—*Current records, Florida reef tract—(Continued)*

Date, July 1914	Time of day	Current: Velocity: Cm. per second	Current: Velocity: Knots per hour	Current: Direction	Wind	Tide
Station No. 14, Half a mile east of Pulaski buoy, Tortugas Lat. 24° 41.6′ N., Long. 82° 45.8′ W. (see accompanying chart)						
	h. m. h. m.					
18	6 00– 6 15 a. m.	19.87	0.386	N. 20° W.	N.E. light	Ebbing
18	7 00– 7 15	21.96	.426	N. 60° W.	N.E. light	Ebbing
18	8 00– 8 15	20.20	.392	N.	N.E. light	Ebbing
18	9 00– 9 15	22.40	.435	N.	N.E. light	Ebbing
18	10 00–10 15	14.37	.279	N.	N.E. light	Ebbing
18	11 00–11 15	8.76	.169	N. 10° E.	N.E. light	Ebbing
18	12 00–12 15 p. m.	15.58	.302	S.	N.E. light	Flowing
18	1 00– 1 15	23.61	.458	S.	N.W. squalls	Flowing
18	2 00– 2 15	28.89	.560	S.	N.W.	Flowing
18	3 00– 3 15	22.18	.430	S. 30° W.	N.E. heavy	Flowing
18	4 00– 4 15	24.38	.473	S. 30° W.	N.E. light	Flowing
18	5 00– 5 15	18.99	.369	S. 30° W.	N.E. light	Flowing
Station No. 15, 1 mile south of south point of Key Biscayne, Flagler Channel, depth of water 17 feet, meter down 6 feet Lat. 25° 38.8′ N., Long. 80° 09.6′ W. (see U. S. C. & G. S. chart 166)						
October	*h. m. h. m.*					
8	6 30– 6 45 a. m.	39.98	0.776	N. 20° E.	N.N.E. light	Flowing
8	7 30– 7 45	56.42	1.095	S. 80° E.	N.N.E. light	Flowing
8	8 30– 8 45	61.46	1.192	S. 80° E.	N.N.E. light	Flowing
8	9 30– 9 45	47.78	.927	S. 80° E.	E. light	Flowing
8	10 30–10 45	32.64	.633	S. 80° E.	E. light	Flowing
8	11 30–11 45	9.5	.185	S. 50° E.	E. light	Flowing
8	12 30–12 45 p. m.	35.18	.682	N. 70° W.	E. light	Ebbing
8	1 30– 1 45	41.78	.811	N. 70° W.	E. light	Ebbing
8	2 30– 2 45	41.90	.813	N. 70° W.	E. light	Ebbing
8	3 30– 3 45	45.10	.875	N. 70° W.	E. light	Ebbing
8	4 30– 4 45	29.54	.573	N. 50° W.	E. light	Ebbing
8	5 30– 5 45	5.30	.103	S. 10° W.	E. light	Ebbing
Station No. 16, 2 miles south of south point of Key Biscayne; depth of water 14 feet; meter down 6 feet Lat. 25° 37.8′ N., Long. 80° 09.6′ W. (see U. S. C. & G. S. chart 166)						
	h. m. h. m.					
9	6 00– 6 15 a. m.	28.10	0.545	N. 40° E.	E. light	Flowing
9	7 00– 7 15	41.66	.808	S. 70° E.	E. light	Flowing
9	8 00– 8 15	48.38	.939	S. 70° E.	E. light	Flowing
9	9 00– 9 15	46.58	.904	S. 70° E.	E. light	Flowing
9	10 00–10 15	29.66	.575	S. 70° E.	E. light	Flowing
9	11 00–11 15	21.14	.410	S. 60° E.	E. light	Flowing
9	12 00–12 15 p. m.	10.34	.201	N. 50° W.	E. light	Ebbing
9	1 00– 1 15	17.54	.340	N. 70° W.	E. light	Ebbing
9	2 00– 2 15	41.06	.797	N. 70° W.	E. light	Ebbing
9	3 00– 3 15	48.74	.946	N. 70° W.	E. light	Ebbing
9	4 00– 4 15	44.34	.860	N. 60° W.	E. light	Ebbing
9	5 00– 5 15	12.02	.233	N. 40° W.	Not given	Ebbing
9	6 00– 6 15	4.16	.081	S. 20° W.	Not given	Ebbing

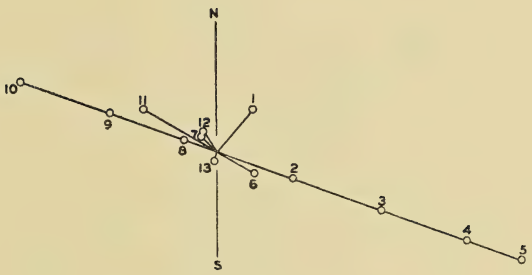
TABLE 2—*Current records, Florida reef tract—(Continued)*

Date, October 1914	Time of day	Current			Wind	Tide
		Velocity		Direction		
		Cm. per second	Knots per hour			
	h. m. h. m.					
Station No. 17, entrance to New Government Cut, off Miami; depth of water 24 feet; meter down 8 feet Lat. 25° 45.8′ N., Long. 80° 08.2′ W. (see U. S. C. & G. S. chart 166)						
11	9 00– 9 15 a. m.	130.94	2.530	S. 70° E.	E. moderate	Flowing
11	10 00–10 15	128.42	2.491	S. 70° E.	E. moderate	Flowing
11	11 00–11 15	123.42	2.394	S. 70° E.	E. moderate	Flowing
11	12 00–12 15 p. m.	109.82	2.131	S. 30° E.	E. moderate	Flowing
11	1 00– 1 15	99.86	1.937	S. 30° E.	E. light	Flowing
11	2 00– 2 15	22.58	.438	S. 50° E.	E. light	Flowing
11	3 00– 3 15	106.58	2.068	N. 30° W.	E. light	Ebbing
11	4 00– 4 15	112.46	2.182	N. 30° W.	Not given	Ebbing
11	5 00– 5 15	108.50	2.105	N. 50° W.	Not given	Ebbing
11	6 00– 6 15	101.78	1.975	N. 50° W.	Not given	Ebbing
	h. m. h. m.					
Station No. 18, Center of Norris cut, east side of Biscayne Bay, depth of water 12 feet; meter down 5 feet Lat. 25° 45.3′ N., Long. 80° 08.4′ W. (see U. S. C. & G. S. chart 166)						
25	6 00– 6 15 a. m.	31.82	0.617	N. 40° W.	S.E. light	Ebbing
25	7 00– 7 15	13.83	.268	N. 40° W.	S.E. light	Ebbing
25	8 00– 8 15	6.74	.131		S.E. light	Ebbing
25	9 00– 9 15	33.14	.623	S. 50° E.	S.E. light	Flowing
25	10 00–10 15	41.66	.808	S. 70° E.	S.E. light	Flowing
25	11 00–11 15	49.34	.957	S. 70° E.	S.E. light	Flowing
25	12 00–12 15 p. m.	46.34	.899	S. 70° E.	S.E. light	Flowing
25	1 00– 1 15	39.22	.761	S. 60° E.	S.E. light	Flowing
25	2 00– 2 15	13.94	.270	S. 60° E.	S.E. light	Flowing
25	3 00– 3 15	22.58	.438	N. 50° W.	S.E. light	Ebbing
25	4 00– 4 15	42.14	.818	N. 50° W.	S.E. light	Ebbing
25	5 00– 5 15	47.06	.913	N. 50° W.	S.E. light	Ebbing
25	6 00– 6 15	47.06	.913	N. 50° W.	S.E. light	Ebbing
November	*h. m. h. m.*					
Station No. 19, Center of Bear Cut, east side of Biscayne Bay, depth of water 18 feet, meter down 7 feet Lat. 25° 43.9′ N., Long. 80° 09.1′ W. (see U. S. C. & G. S. chart 166)						
11	7 00– 7 15 a. m.	59.14	1.147	S. 60° W.	N.W. light	Ebbing
11	8 00– 8 15	26.58	.516	S. 60° W.	N.W. light	Ebbing
11	9 00– 9 15	18.44	.358	S. 60° W.	N.W. light	Ebbing
11	10 00–10 15	61.78	1.202	N. 60° E.	N.W. light	Flowing
11	11 00–11 15	62.55	1.213	N. 60° E.	N.W. light	Flowing
11	12 00–12 15 p. m.	61.23	1.188	N. 60° E.	N.W. light	Flowing
11	1 00– 1 15	57.93	1.124	N. 60° E.	N.W. light	Flowing
11	2 00– 2 15	35.71	.693	N. 60° E.	N.W. light	Flowing
11	3 00– 3 15	7.88	.153	N. 60° E.	N.W. light	Flowing
11	4 00– 4 15	31.64	.614	S. 60° W.	N.W. light	Ebbing
11	5 00– 5 15	43.74	.849	S. 60° W.	N.W. light	Ebbing
11	6 00– 6 15	57.82	1.122	S. 60° W.	N.W. light	Ebbing

The data here recorded and the notes on them constitute a bit of preliminary information on the currents of the Florida Keys. A series of current measurements along Hawk Channel at different seasons is needed for a more adequate understanding of some of the phenomena exhibited in the Florida Key region.



STATION 15, OCT. 8



STATION 16, OCT. 9

FIG. 12—Current rosettes for stations 15 and 16. Currents are represented as flowing toward station. Sequence of readings, which were hourly, is shown by numbers beginning with 1. Velocity is indicated by length of line, scale: 1 mm.=4 cm. per second. 1 cm. per second=0.0194 knots per hour.

The direction of the current as given in the tables is the direction from which the current flows, that is, the direction indicated as S.50°W. means that the current flows from S.50°W. to N.40°E. The directions are magnetic and have not been corrected for magnetic variation.

For all stations, appropriate current rosettes have been constructed. In each rosette the currents are represented as moving toward a point, the one at which the boat was anchored. The sequence of the readings is shown by the sequence of the numbers beginning with "1." The velocity is in-

dicated by the length of the line. The scale for the text figures is 1 mm.=4 cm. per second; 1 cm. per second=0.0194 knots per hour; 52 cm. per second=approximately 1 knot per hour, or, a line 13 mm. long means a current of one knot per hour.

The position of each station is given and, except for the Tortugas, reference is made to the appropriate U. S. Coast and Geodetic Survey chart. For the Tortugas area, in which 8 stations were occupied, the positions of

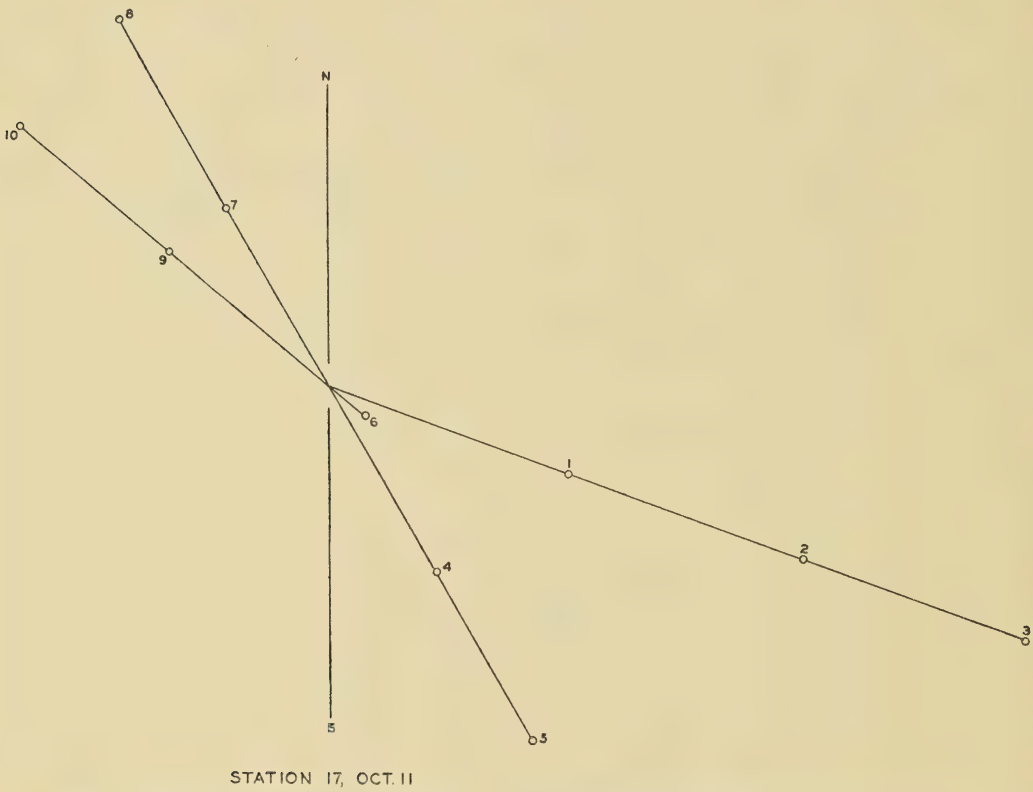


FIG. 13—Current rosette for station 17. Currents are represented as flowing toward station. Sequence of readings, which were hourly, is shown by numbers beginning with 1. Velocity is indicated by length of line, scale: 1 mm.=4 cm. per second. 1 cm. per second= 0.0194 knots per hour.

the stations have been plotted on the Coast and Geodetic Survey chart entitled, "Tortugas Harbor and Approaches," C.& G.S. No. 471a, which is herewith reproduced. The current rosettes for the different stations have been printed in red on the chart. There is one rosette for each station, except station No. 7, for which there are two, and for station No. 9, for which there are four rosettes.

On the Tortugas chart I have had drawn a wind rosette for Key West for the year 1902 prepared by Cox.[1] I have discussed the conformity of

[1] R. D. Salisbury, Physiography, 611, 1909.

the outlines of the Tortugas to winds and currents in the paper cited below.[1] The arc in the northeast part of the area is bowed toward the direction from which the prevailing winds blow. The southwest end of the bank above which Loggerhead Key rises is bent westward in conformity to the currents recorded at stations 7 and 13.

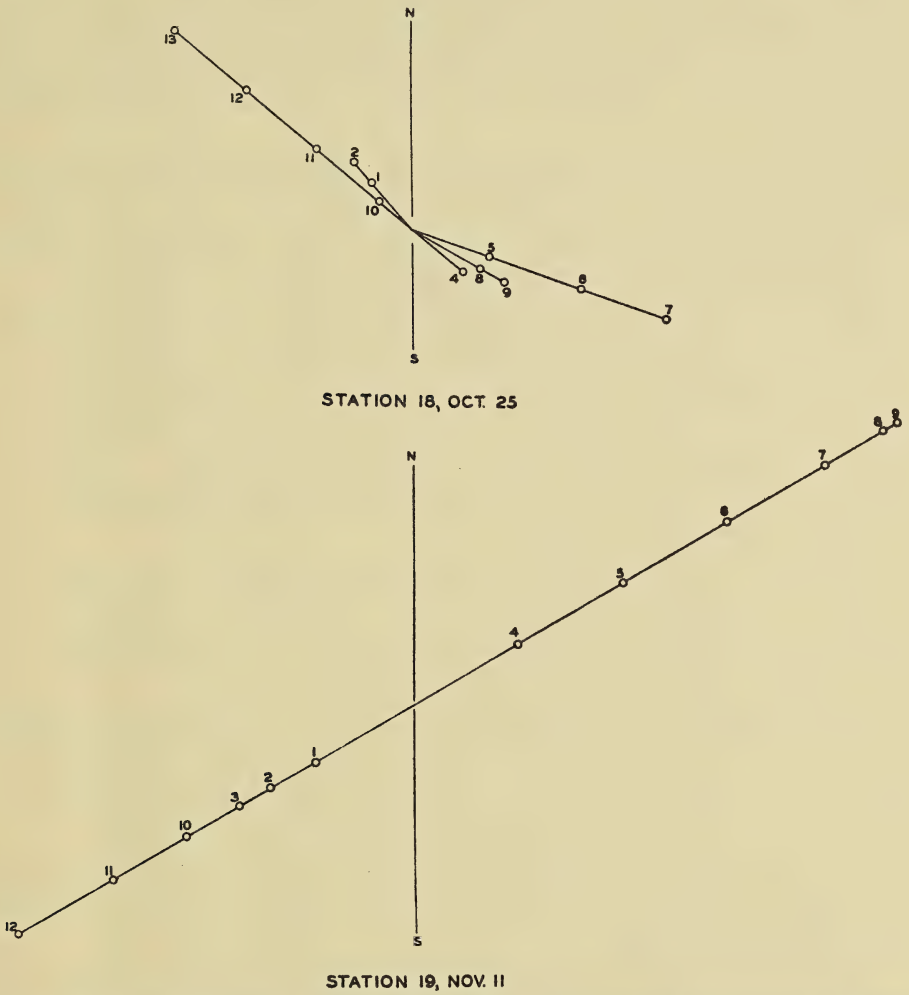


FIG. 14—Current rosettes for stations 18 and 19. Currents are represented as flowing toward station. Sequence of readings, which were hourly, is shown by numbers beginning with 1. Velocity is indicated by length of line, scale: 1 mm.=4 cm. per second. 1 cm. per second=0.0194 knots per hour.

[1] T. W. Vaughan, *The building of the Marquesas and Tortugas Atolls and a sketch of the geologic history of the Florida Reef Tract,* Carnegie Inst. Wash. Pub. No. 182, 55-67, 1914.

NOTES ON CURRENT OBSERVATIONS, FLORIDA KEYS, JUNE, OCTOBER, NOVEMBER, 1914

By Frank J. Haight[1]

Observations were made at 19 stations, numbered 1 to 19, inclusive. At stations, 1, 2, and 3 the observational periods were too brief for the results to serve as an index to the nature of the current movements. At each of the remaining stations, with the exceptions of those numbered 5 and 9, approximately 1/2 day of observations was obtained. At station 5 the observational period was about 1/4 day and at station 9 it was about 2 1/2 days.

The times of slack water and the times, velocities and directions of the strengths of flood and ebb, as derived from plottings and reductions of the observations, have been compiled in tabular form. The times have been referred to the times of high water and low water at Key West and the velocities have been corrected to approximate mean values by the application of factors which in each case represent the ratio of the mean range of tide to the range during the period when the observations were taken.

The tabular values serve as a rough index to the general trend of the currents at the various stations, the series being too short to admit of the derivation of precise values. However, they constitute, with one exception, the only current information available for the locations occupied and are, therefore, of considerable value.

At station 4 in Hawk Channel, the observed velocities were small, the maximum being slightly less than 0.3 knot. Observations at stations 5 and 6 indicate that the tidal currents north and south of the viaduct at Knights Key attain a velocity approximating 2 knots.

Results for stations 7 to 14, inclusive, in the vicinity of Dry Tortugas, indicate that tidal currents having velocities about 1 1/2 knots at strength set through the passages between the keys, their direction being generally northward on the flood and southward on the ebb. At stations 7 and 13, southward of the keys, a nontidal set to the westward is indicated.

At stations 15 and 16, south of Key Biscayne, currents of between 1 and 1 1/2 knots, setting into and out of Biscayne Bay on the flood and ebb, respectively, were observed.

Flood and ebb velocities exceeding 3 knots are indicated in Miami Entrance, station 17. The results of a two-day series of observations taken in this Entrance in 1929 by the Coast and Geodetic Survey show a mean velocity of about 2 1/2 knots at strength of flood and about 3 knots at strength of ebb.

At station 18 in Norris Cut and station 19 in Bear Cut, passages into Biscayne Bay southward of Miami Entrance, velocities somewhat in excess of 1 knot were observed.

[1] Associate Mathematician, U. S. Coast and Geodetic Survey.

TABLE 3—*Currents: Recapitulation of results, as reported by Dr. T. Wayland Vaughan.*

General locality, Florida Reefs. Referred to High and Low Water at Key West, Florida. Velocities corrected for range of tide.

Station Number	Location	Observations				Slack	Flood Strength			Flood Duration	Slack	Ebb Strength			Ebb Duration
		Date	Period	Method	Depth		Time	Direction (true)	Velocity			Time	Direction (true)	Velocity	
		1914	*Days*		*Feet*	*Hours after L. W.*	*Hours before H. W.*	°	*Knots*	*Hours*	*Hours after H. W.*	*Hours before L. W.*	°	*Knots*	*Hours*
4	Off Rodriguez Key, Hawk Channel 25° 02.2′ N., 80° 26.2′ W.	June 5	½	Ekman Meter	.....	2.0	0.0	200	0.3	.....	3.0	.....	.....	.....	.....
5	North side of viaduct, Knight's Key 24° 42.5′ N., 81° 08.2′ W.	June 6	¼	do.	.....	*	.....	.....	.....	.....	.....	.....	.....	.....	.....
6	South side of viaduct, Knight's Key 24° 42.2′ N., 81° 08.2′ W.	June 6–7	½	do.	.....	1.8	0.4	355	1.2	.....	3.0	0.8	160	2.2	.....
7	South side of Loggerhead Buoy 24° 36.6′ N., 82° 57.5′ W.	June 15 & 21	1	do.	18	†	.....	.....	.....	.....	.....	.....	.....	.....	.....
8	Off White Shoal, Tortugas, Bet. Buoys C 3 & N 4 24° 37.7′ N., 82° 54.0′ W.	June 15	½	do.	.....	2.8	0.5	10	1.4	.....	2.2	0.5	190	1.4	.....
9	Off east side of Loggerhead Key, Tortugas 24° 38.1′ N., 82° 54.8′ W.	June 22–25	2½	do.	18	0.3	2.5	23	1.7	6.9	0.4	2.6	222	1.0	5.5
10	Off west side of Loggerhead Key, Tortugas 24° 38.2′ N., 82° 55.4′ W.	June 23	½	do.	4	‡	.....	.....	.....	.....	.....	.....	.....	.....	.....
11	S.E. Channel, Bet. Bush Key & Buoy N 2, Tortugas 24° 37.4′ N., 82° 50.7′ W.	June 24	½	do.	.....	−1.0	.....	.....	.....	.....	0.2	3.8	135	0.8	.....
12	Northwest Channel, Tortugas 24° 41.9′ N., 82° 52.8′ W.	June 25	½	do.	.....	0.3	.....	.....	.....	.....	−1.0	3.3	180	0.7	.....
13	Near Buoy N 8, Tortugas 24° 35.1′ N., 82° 54.1′ W.	July 19	½	do.	.....	§	.....	.....	.....	.....	.....	.....	.....	.....	.....
14	One-half mile E. of Pulaski Buoy, Tortugas 24° 41.6′ N., 82° 45.8′ W.	July 18	½	do.	.....	−1.2	3.7	20	0.7	.....	.....	4.5	170	0.6	.....
15	One mile S. of Key Biscayne, Flagler Channel 25° 38.8′ N., 80° 09.6′ W.	Oct. 8	½	do.	.....	.....	2.9	280	1.4	.....	0.5	2.1	110	1.2	.....
16	Two miles S. of Key Biscayne 25° 37.8′ N., 80° 09.6′ W.	Oct. 9	½	do.	.....	.....	3.7	290	1.2	.....	−0.3	2.2	113	1.2	.....
17	Miami, Entrance 25° 45.8′ N., 80° 08.2′ W.	Oct. 11	½	do.	.....	.....	4.6	290	3.6	.....	0.5	3.3	150	3.2	.....
18	Center of Norris Cut, Biscayne Bay 25° 45.3′ N., 80° 08.4′ W.	Oct. 25	½	do.	.....	0.5	3.4	290	1.1	.....	−0.4	2.9	130	1.0	.....
19	Center of Bear Cut, Biscayne Bay 25° 43.9′ N., 80° 09.1′ W.	Nov. 11	½	do.	.....	0.5	4.1	240	1.4	.....	0.0	.....	.....	.....	.....

Remarks: * Currents of 1.6 kn. setting 190° and of 1.7 kn. setting 350° were observed.

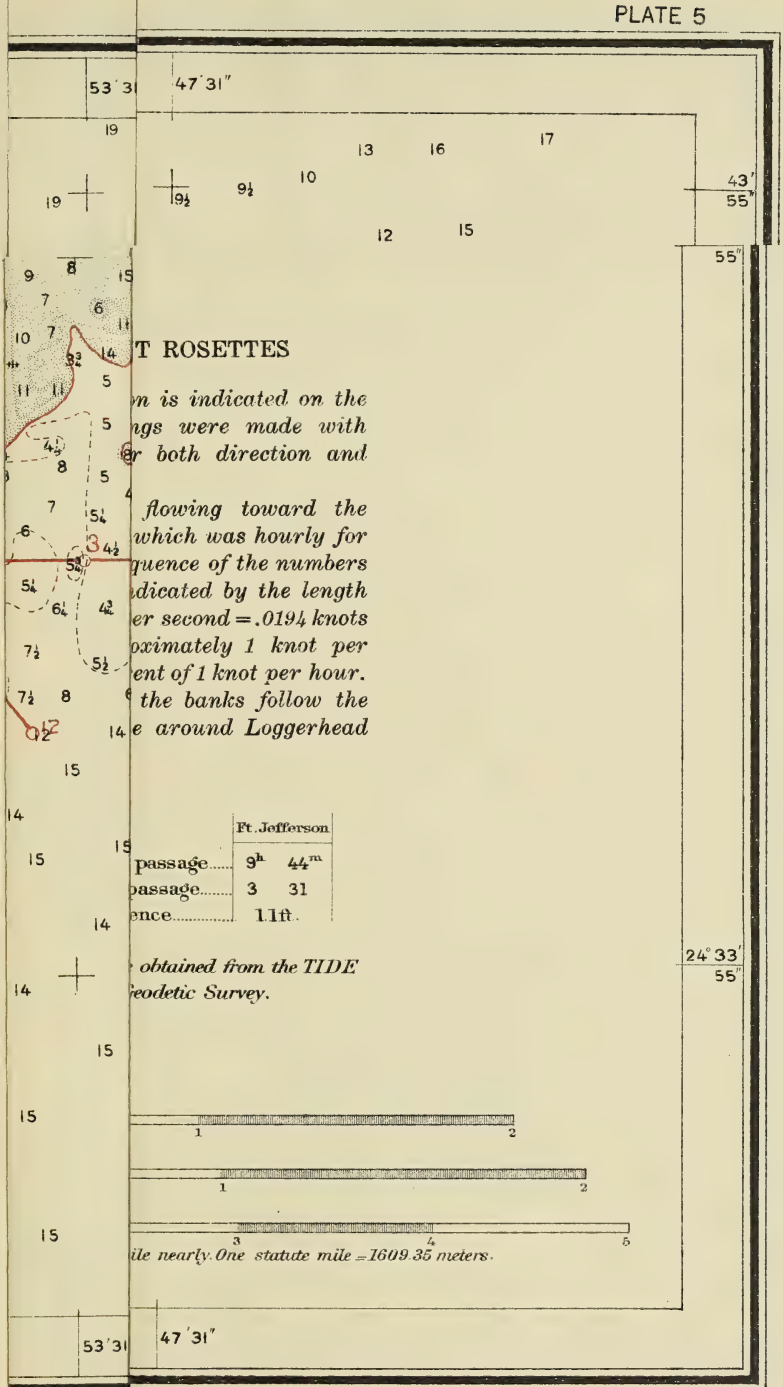
† Observed current irregular, mostly westward, velocity varied from 0.0 kn. to 1.2 kn., direction from 140° clockwise to 340°, maximum velocity 1.2 kn., 320°.

‡ Observed current irregular, velocity varied from 0.0 kn. to 0.5 kn., direction from 190° through west to 10°, maximum velocity 0.5 kn., 290°.

§ Observed current westward, velocity varied from 0.2 kn. to 0.7 kn., direction from 270° to 320°, maximum velocity 0.7 kn., 270°.

NOTE.—At stations 1, 2, and 3 the observations were too brief to indicate the nature of the current.

PLATE 5



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obtained from the TIDE
Geodetic Survey.

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TO T

Station 9
June 23
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Station 12
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July 18
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June 28
Station 8, June 15
Station 11
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Station 6
June 22
Station 7
June 15
Station 7
June 21
Station 13
July 19
Loggerhead Key
North Key
North Key Harbor
Northeast Key
Middle Key
East Key
Sand Key
Long Key
Bird Key
Hospital Key
Bush Key
Garden Key
Bird Key
Santa Lucia Reef
Middle Ground
Rebecca Shoal
Rock Key
Rock Key
Rock Key

PAPERS FROM TORTUGAS LABORATORY, VOL. XXIX

V

INTERSEXUALITY IN THE CRUSTACEA

By Hugh H. Darby

With one plate

[Issued December 9, 1935.]

INTERSEXUALITY IN THE CRUSTACEA

In 1920 Morgan raised the question of intersexuality in the Crustacea by calling attention to the variation in the abdomen widths in fiddler crabs. The young fiddler crab passes through an indeterminate period in which the abdomen of both sexes is narrow. At a definite stage in development, the abdomen of the female widens to cover the entire ventral surface, while the male abdomen remains a narrow strip in the middle. About the time that this change in the female takes place, one or both of the first pair of walking legs of the male becomes modified into a large claw or fiddle. There are, therefore, two independent types of sexually significant change: in the female, widening of the abdomen without differentiation of the chelæ; and in the male, differentiation of the chelæ without widening of the abdomen.

Morgan's main subject of study was the abdominal change in the female, although some incidental information on chelæ was included. He found that a few mature females had abdomens much narrower than the average. These females he termed intersexes. Rathbun (1921) criticized this suggestion and stated that these were only the extreme variants of the normal developing crab. Huxley (1924) made a mathematical analysis of the data and came to the same conclusion. He writes (1932), ". . . that these were merely the extreme minus variants for the normal variation curve of female abdomen growth and that the supposition of intersexuality was uncalled for." Both Rathbun's and Huxley's methods describe a state—even though Huxley's is mathematical description—and say nothing of the factors responsible for variation. Morgan, on the other hand, was interested in the cause of variation, and his suggestion of the existence of intersexuality is in no way invalidated by the findings of the other workers. That clear-cut cases of intersexes do exist in the Crustacea, and in particular in the fiddler crabs, will be shown in this paper.

FEMALE TO MALE INTERSEXES

While Morgan centered his attention on the abdomen, it seemed to me worth while to attempt to find females that carried male claws. Since in the female the claws are normally equal and small, it was thought that any abnormal male hormone formation should lead to two large chelæ or fiddles. In some 9000 specimens of *Uca pugilator* collected at Beaufort, North Carolina, 12 females were obtained with two fiddles (Plate I). No female crab was found with one large chela. The normal female chelæ are small and relatively undifferentiated, but in these intersexes they were typical male appendages. The abdomens were fully widened and typically female. Moreover, these individuals were always found feeding with the males, in contrast to the other small-clawed females which stayed close to their holes.

The concept of humoral distribution of a substance (possibly male sex hormone) causing large claw differentiation, as suggested in my work on *Crangon* (Darby, 1934), would seem to be applicable to these females, where differentiation is bilateral. Furthermore, Morgan (1923) has observed that even in the male, many individuals are so symmetrical at early stages that the original differentiation of the fiddle affects both sides. It is only by the accidental loss of one chela that they later become asymmetrical.

MALE TO FEMALE INTERSEXES

A search was also made for a crab with a typical male abdomen and normal male reproductive appendages, yet with small claws. In the large collections made at Beaufort, North Carolina, none was found; but at Charleston, South Carolina, one animal with these characteristics came to light. Morgan also reported such a male adult, but did not consider it an intersex. The claws of my specimen were typically those of a female, both in size, shape and abundance of setæ. It may be suggested that this crab had lost its fiddle and was regenerating a new one; but this can not be the case, for two reasons. (1) If the fiddle is removed, the claw regenerated at the next moult is much reduced in size, but morphologically a fiddle. Morgan stated this fact in 1919, and I have recently been able to obtain the same results on sixty specimens. (2) The pigmentation at the joints of regenerating claws is much lighter than in old claws; and the claws of this crab were of normal color.

It is therefore clear that with the abdomen as a criterion of sex, each sex can show modifications in the direction of the other. The linkage of these two secondary sexual characters—abdomen and claws—is therefore incomplete.

SYMMETRY IN MALES

Morgan (1923), in describing 165 crabs of 2 to 4 mm. carapace width, gives their distribution as follows:

9 crabs with unequal claws
56 crabs with both claws large
100 crabs with both claws small

The first two groups were obviously males, with a possibility of, let us say, one female in the second group. The third group must have included both sexes. Now, if we assume that the last group is 50 per cent male, and allow an error of 10 per cent either way, we see that out of $9+56+40=105$ males (or $9+56+60=125$ males), 56, or about half, had differentiated both claws. If we assume that 50 per cent of all crabs collected were males, then 67 per cent showed bilateral differentiation. In other words, the two sides in the developing fiddler crab are much more nearly symmetrical than the

extreme asymmetry of the adult male would suggest. In *Crangon,* I have shown (Darby 1934) that if both sides are made equal in development, the animal will differentiate two large chelæ. The proof of this possibility in *Uca* must lie either in the experimental production of such an animal, or its discovery in nature. As the former was impossible on account of the short season at my disposal, the latter was resorted to. In 10,000 specimens, 20 males were found with varying approximations of symmetry through partial modification of the small chela into a fiddle (Plate I). As from my observations of the behavior of the normal male, feeding is accomplished exclusively with the small chela, these partially symmetrical males must be seriously handicapped in obtaining food. Since the handicap would increase with increasing modification of the utilitarian small chela, it is not surprising that not a single perfectly symmetrical adult male could be found.

In a later paper, some of the mechanics of differentiation of the chelæ in *Crangon* will be described in detail. Suffice it to say here that the process of differentiation of the large chela is not continuous throughout the intermolt period, and that it has been possible to locate the actual time at which it takes place. It is only in line with other researches to suppose that some definite chemical substance is responsible for the formation of secondary sexual characters—in other words, that the hormone concept can be extended to include the Crustacea. The still evasive link between the chromosome and its final expression, the adult morphological structure, seems to be susceptible of study from this line of approach.

Part of these researches were conducted at the Laboratory of the U. S. Bureau of Fisheries in Beaufort, North Carolina. I wish to acknowledge the assistance of Mr. Frank T. Bell, Commissioner of Fisheries, and the helpful cooperation of Dr. Herbert F. Prytherch, Director of the Laboratory.

The specimens photographed in this paper are now in the collection of the U. S. National Museum, Washington, D. C.

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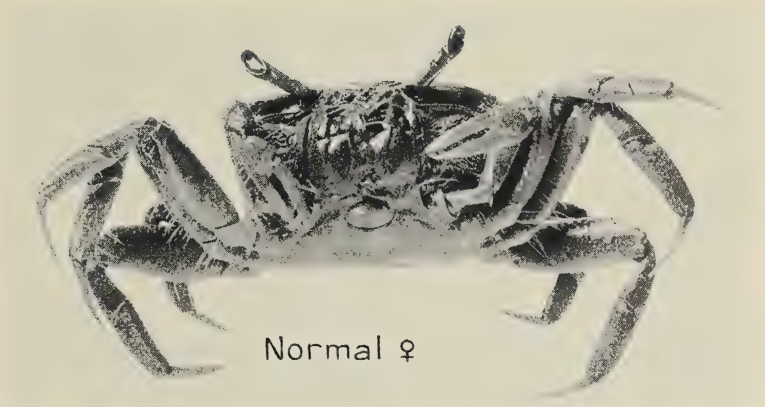
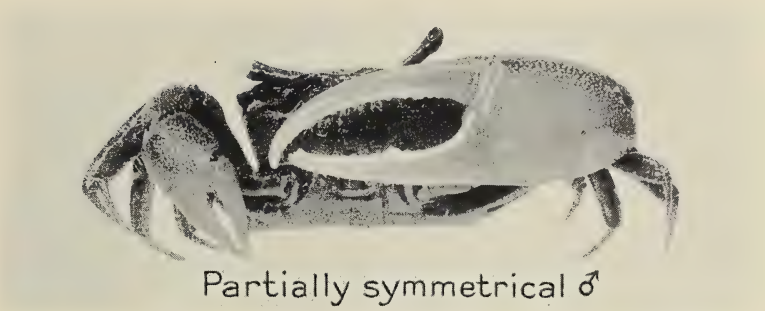
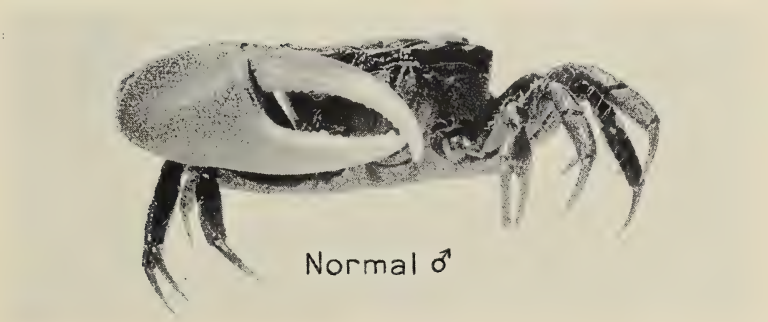
♀ Intersex



Normal ♀



Partially symmetrical ♂



Normal ♂

PAPERS FROM TORTUGAS LABORATORY, VOL. XXIX

VI

THE MECHANISM OF CHELA DIFFERENTIATION IN THE CRUSTACEA

By Hugh H. Darby

[Issued December 9, 1935.]

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THE MECHANISM OF CHELA DIFFERENTIATION IN THE CRUSTACEA

INTRODUCTION

The interesting phenomenon of reversal of asymmetry in Alpheid shrimps, first described by Przibram in 1901, was further investigated in *Crangon* during the summers of 1933 and 1934, and has proved fruitful as a means of approach to some of the problems of development. These crustaceans normally possess a marked asymmetry of the chelæ, having one large chela or snap-claw and one small chela or pinch-claw, with a random distribution as to side. The snap-claw is a totally different type of structure from the pinch-claw and is used differently. Przibram (1901, 1902) showed that when the snap-claw was removed, the pinch-claw differentiated into a snap-claw at the next molt, and a new pinch-claw regenerated at the site of the former snap-claw. Hence, the pinch-claw is capable of differentiation, although normally it does not do so. This observation has been confirmed by Wilson (1903), Zeleny (1905), Darby (1934) and Dawes (1934).

In a recent work (1931), Przibram suggested that the chela which develops into a snap-claw does so by virtue of an earlier start in growth than the other. Unfortunately this publication had not come to my attention in 1934; and in arriving at the same conclusion on the basis of experimental evidence, I did not refer to it. This oversight I beg to correct here.

The present paper will present further evidence for my hypothesis that differentiation of chelæ beyond the simplest type in *Crangon* and related Crustacea is due to the discontinuous elaboration of a definite chemical entity, comparable with the male sex hormone of other animals. The conditions under which this substance acts and its modifying effects on a basic and older (in an evolutionary sense) structure will be outlined. In passing, some of J. S. Huxley's ideas on heterogonic growth will be shown to be inapplicable, owing to the non-identity of growth and differentiation.

METHODS

The method of rearing *Crangon* described in an earlier paper (Darby, 1934) was adhered to in the present experiments, with one improvement. Several small algæ from the rocks under which the organisms live were added to the culture dishes. The *Crangon* feed on these algæ and seem to thrive much better with this addition to their diet.

In the process of irradiation with radium, the chamber had to be so small (to keep the organisms at a constant distance from the radium needle) that running water was passed through it to supply adequate oxygen. The animals were not fed during irradiation, but immediately before and after.

Unless otherwise specified, the experiments were run at room temperature, which varied between 25.5° and 29° C., without violent fluctuations. Although the importance of controlling the temperature more closely than this was fully realized, only one incubator was available.

SNAP-CLAW DIFFERENTIATION IN RELATION TO THE MOLT

I have already postulated (1934) that at some point between molts a substance "B" is liberated, which is responsible for snap-claw formation, is used up during the process, and without which *Crangon* is unable to differentiate a large chela. To support this hypothesis, it is necessary to show that during certain portions of the intermolt period, the organism produces only pinch-claws. The experimental procedure on this point con-

TABLE 1—*Results of removing the large chela soon after a molt, followed by removal of the small chela at stated intervals before the next molt.*

Time of removal of small chela	Total number of organisms	Chelæ regenerated				
		1 large and 1 small	Both small	One small only	One large only	No chelæ
7th day before molt....	10	9	1	..	..	..
6th day before molt....	10	4	5	..	1	..
5th day before molt....	8	2	6	..	..	..
4th day before molt....	2	..	2	..	..	..
3d day before molt....	5	..	1	4	..	..
2d day before molt.....	5	..	..	4	..	1
1st day before molt.....	4	..	..	3	..	1

sisted in removing the snap-claw one or two days after a molt,[1] then leaving an interval of three or more days before removing the pinch-claw. With the snap-claw removed, the remaining pinch-claw starts to differentiate into a snap-claw. This development of the pinch-claw into a snap-claw should drain the body of all the available substance "B"; and should this differentiating chela now be removed, the body should fail to produce any large chela at all. When *Crangon* was treated in this manner, it soon became evident that during the last four days before molting, the animal produced one or even two pinch claws, but no snap-claw. In other words, a regenerating pinch-claw was present for several days before molting without being differentiated into a snap-claw. The results of this experiment are detailed in table 1.

In the foregoing experiment, the large chela was removed first, so that the small chela might serve as a drain to exhaust substance B. In the next experiment, the order of removal was reversed, that is, the small chela was

[1] The average intermolt period (at *ca.* 27° C.) is about 11 days.

taken off first, and the large chela at stated intervals afterward. Now the tendency, on removal of the small chela, will be for the regeneration of another small chela in the same place. As the large chela is in position, there should be no large demand for substance B by a regenerating chela, as there was in the last experiment. Table 2 shows that the production of a large chela under this latter system can take place up to 4 days before molting, and in a partial degree up to 3 days before molting. It substantiates the first experiment in showing that on the last few days preceding a molt, only pinch-claws are grown; and that even during the 2 days previous to that, only a small percentage of the animals can differentiate a snap-claw.

TABLE 2—*Results of removing the small chela soon after a molt, followed by removal of the large chela at stated intervals before the next molt.*

Time of removal of large chela	Total number of organisms	Chelæ regenerated				
		1 large and 1 small	Both small	One small only	One large only	No chelæ
7th day before molt.....	91	86	5	..	..	..
6th day before molt.....	51	44	5	2	..	..
5th day before molt.....	27	15	5	3	4	..
4th day before molt.....	13	3	3	2	5[1]	..
3d day before molt.....	5	..	..	4	1[1]	..
2d day before molt.....	5	..	..	5	..	..
1st day before molt.....	4	..	..	1	..	3

[1] Very poorly differentiated.

It is clear from table 1, where the utilization of B by a regenerating chela was complete, that the substance responsible for snap-claw formation must have disappeared by the fourth day before molting, and must therefore have been elaborated previous to that time. In an 11-day period between molts, this leaves the first 7 days within which it must have been produced. I have already shown that at this temperature (*ca.* 27° C.) the small chela needs a start of from 36 to 48 hours in order to become a snap-claw. It follows that if this handicap of 48 hours at the start is deducted from the 7 days, we have left a 5-day period, approximately midway between molts, within which differentiation must take place. The aim of the above experiments has been accomplished, in that differentiation has been shown not to occur during the first and last portions of the intermolt period. In other words, the possible differentiation period has been cut from 11 days to 5. Further experiments in this period will show whether the time limit can be narrowed still further.

EFFECTS OF GAMMA RADIATION ON GROWTH AND DIFFERENTIATION OF CHELÆ

To locate more closely the exact time of differentiation, irradiation of the animals with gamma rays from radium was resorted to. Since previous work had shown that one of the effects of this type of radiation is the retardation of metabolism (Obreshkove and King, 1932; Darby, 1934), it was thought that growth rate might be similarly affected. Moreover, the results of some preliminary experiments along this line tended to show that the differentiation process might be suppressed.

The first experiment with radium was planned to demonstrate the presence of any marked effect on the organism. The small chela was taken off, and irradiation begun with 7.1 mg. of Ra at a distance of 2 cm. After 36 hours of irradiation, the animals were removed from the influence of the radium and the large chela taken off immediately. The only effect noticed

TABLE 3—*Effect of irradiation with radium on chela reversal.*
(7.1 mg. of Ra, at 2 cm. distance, for 36 hours)

	Number of cases	Per cent reversed	Per cent died
Irradiated animals	29	0	10
Same animals, next molt, not irradiated	16	53	6
Unirradiated controls	54	56	11

was the slowing up of the development of the small chela, as shown in table 3 by the absence of reversal. As soon as these animals had molted, they were subjected to the same experimental procedure again, with the irradiation omitted. Another set of animals that had received no irradiation at all were run as controls, to give the normal expectancy of reversals.

From the above figures it is clear that 36 hours of irradiation prevented the reversal of sides which would normally have happened in about 50 per cent of the animals.

This effect was only temporary, as the same individuals behaved in a perfectly normal manner after the next molt. The death rate compared favorably with that in the controls, and during the next molt was even lower, though not significantly so.

The next step was to lengthen the period of irradiation to 44 hours and also to lengthen the period between the removal of the small chela and the large, in order to compensate for the retarding effect of irradiation on growth rate and so favor the probability of reversal. The results of this procedure are given in table 4.

TABLE 4—*Effect of irradiation with radium on chela reversal.*
(12.16 mg. of Ra, at 2 cm. distance, for 44 hours)

	Number of cases	Per cent reversed	Per cent died
Irradiated animals	23	0	35
Same animals, next molt, not irradiated	13	75	8
Unirradiated controls	36	74	3

Here again, the animals have been definitely prevented from reversal; in other words, the development of the pinch-claw has been retarded to such an extent that the snap-claw still has the advantage in spite of a 44-hour handicap. Irradiation had not injured the survivors, as they showed good agreement after the next molt with the controls. However, that some harmful effect was coming into play was shown by the high death rate of 35 per cent following irradiation.

One more experiment of this type was tried, in which the period of exposure was extended to 48 hours, and the chelæ removed 48 hours apart, starting with the small one as usual. These results are to be found in table 5.

TABLE 5—*Effect of irradiation with radium on chela reversal.*
(12.16 mg. of Ra, at 2 cm. distance, for 48 hours)

	Number of animals	Per cent reversed	Per cent died
Irradiated animals	56	13	46
Same animals, next molt, not irradiated	19	71	17
Unirradiated controls	23	86	9

These results are similar to those of the previous experiment, except that the irradiation did not retard all of the organisms sufficiently to prevent reversal, and 13 per cent reversed. This type of experimentation obviously can not be extended further. The death rate here was 46 per cent, and there is evidence suggesting an after-effect. There is a reduction of reversals after the next molt as compared with the controls, and a persisting high death rate. The results of all three experiments are summarized in table 6.

The three foregoing experiments, considered in the light of previous findings on the lowering of metabolic rate by irradiation, show that any retardation in the rate of development of the pinch-claw in the course of its race with a regenerating snap-claw results in the retention of the snap-claw by the original side. It has been shown that the time factor can settle the question of which side is to have the snap-claw. That time factor has

now been modified by irradiation in such a way as to lend support to the hypothesis of competition.

In this experiment, 48 hours of irradiation permitted 13 per cent of the animals to show chela reversal on the strength of a 48-hour start. Without the irradiation, the same 13 per cent reversal would have resulted from a start of only 30 hours. Since the difference in time (30 hours without irradiation, 48 hours with it) is an increase of 60 per cent, the growth rate of the pinch-claw must have been reduced to about 3/5 of its original value. Incidentally, by rigid control of temperature it will be possible with this organism to compare irradiation with temperature directly, with reference to their effects on growth rate. This work is now in progress.

TABLE 6—*Effect of irradiation with radium on chela reversal.*

Interval between small and large chela removal	Dosage of Ra in mg. hrs.	Per cent reversal		
		Irradiated animals	Same, next molt, not irradiated	Controls, not irradiated
36 hrs.	7.1 x 36	0	56	53
44 hrs.	12.2 x 44	0	75	74
48 hrs.	12.2 x 48	13	71	86

It will now be interesting to see whether retardation applied to regenerating chelæ at different times in the process will have similar effects, judging by the percentage of reversal. The type of irradiation procedure used for this purpose was to double the amount of irradiation by using 24 instead of 12 mg. of radium, but to halve the time of exposure, allowing 24 (instead of 48) hours. The chelæ were taken off in the same order as before, starting with the pinch-claw, with a 48-hour interval between them. The only variable in these experiments was the actual time at which the animals were irradiated. In one set, the irradiation was done over the first 24 hours, immediately after the removal of the first chela; in the next set, over the second 24 hours, and so on.

The aim of this experiment was to utilize the fact demonstrated in the last three experiments—retardation of the growth of the small chela—to locate the time at which the differentiation process takes place. What we want to know is whether the retardation which results from irradiation during the first 48 hours will occur following irradiation at later periods, and whether it will affect reversal. In other words, at what time, if any, in the intermolt period can irradiation be applied without affecting reversal? From the experiments already reported on chela removal shortly before the molt, we know that in the last few days before a molt, irradiation should not

affect reversal, because differentiation has already taken place. To check this point, irradiation was applied at various times during the intermolt period, and the results are to be found in table 7.

It will be seen that there was a strong inhibition of reversal when irradiation was applied over the first four intervals listed in table 7. From the 72d hour on, however, reversal was not inhibited at all. The portion of the 60 to 84 hour period during which the observed inhibitory effect took place must therefore have been the first half—that is, before 72 hours. When reversal was not inhibited, the pinch-claw must have completed its differentiation into a snap-claw before the irradiation was applied; that is, retard-

TABLE 7—*Effect on chela reversal of irradiation applied at various times in the intermolt period. Pinch-claw removed first, snap-claw 48 hours later.* (24.32 mg. Ra, at 2 cm. distance, for 24 hours)

Period of irradiation (hours)	No. of organisms	Per cent reversed	
		Irradiated animals	Unirradiated controls
0 to 24th	15	17	75
24th to 48th	37	14	75
48th to 72d	24	9	75
60th to 84th	16	13	75
72d to 96th	18	78	75
96th to 120th	20	85	75
120th to 144th	12	92	75
144th to 168th	12	92	75

ation of the growth of the pinch-claw no longer prevented it from winning the race for substance "B," the goal having already been attained. From this it is concluded that differentiation (at a temperature of *ca.* 28° C.) must take place at some time between the 60th and 72d hour; in other words, the process that determines which side is to have the snap-claw is completed within the short space of 12 hours. In the entire 11-day intermolt period, it is at the end of the third day after the removal of the first chela (pinch-claw) or about the seventh day before molting, that this differentiation takes place. I have already shown that it has to occur prior to the fifth day before molting. Now the time can be specified within 12 hours.

A little more explanation of the data may be of assistance in visualizing this developmental mechanism. In table 7, the higher than control figures for the last three days are due to the following considerations. When an interval of 48 hours is allowed between pinch-claw and snap-claw removal, and the animals are not irradiated, only 75 per cent undergo reversal. This leaves 25 per cent unreversed, consisting of those individuals whose snap-claw growth rate is so high that a 48-hour handicap is not sufficient to prevent the large chela from overtaking the small, when both are regener-

ating. Now, if irradiation is applied as late as 96 to 120 hours after pinch-claw removal, the retarding effect is more marked on the snap-claw, since it has only been growing for 48 hours, while the pinch-claw has been growing for 96. The retardation of these extra-fast snap-claws favors the possibility of reversal. This same effect persists in the irradiation periods between 120 and 168 hours. The reversal figures would have been even higher in these cases, had not quite a few of the animals come out with both chelæ small.

There is another method of analyzing these data. The effect on either side can be defined irrespective of the type of chela regenerated, with reference only to the ability of that side to produce a chela at all. Such an analysis is presented in table 8.

TABLE 8—*Effect of irradiation at various times on the regenerative ability of the two sides. (Period of irradiation measured using time of pinch-claw removal as starting point.)*
(24.32 mg. of Ra, at 2 cm. distance)

Period of irradiation	Total No. of animals	Per cent of chelæ regenerated	
		At site of original pinch-claw	At site of original snap-claw
0 to 24th hr.....	18	100	89[1]
24th to 48th hr.....	38	98	92[1]
48th to 72d hr.....	33	73	97
60th to 84th hr.....	18	61	100
72d to 96th hr.....	25	92	60
96th to 120th hr.....	27	93	85
120th to 144th hr.....	13	100	84
144th to 168th hr.....	13	100	84

[1] Irradiation preceded removal of snap-claw.

In table 8 it will be seen that repression of regeneration on the side of the small chela occurred between the 48th and 72d hour and between the 60th and 84th hour. Owing to the fact that no similar damage was produced by irradiating between the 72d and 96th hour, we know that the effective time must have been between the 60th and 72d hour. This period is obviously included in both the 48- to 72-hour and the 60- to 84-hour irradiations, and will account for the effects observed in both. Thus the side from which the small chela had been removed was most susceptible to injury between the 60th and 72d hour of growth. On the large chela side, it would appear that the 72- to 96-hour period was the most susceptible to injury; but it must be remembered that the large chela was not removed until 48 hours after the small one, so that the 72- to 96-hour irradiation period was really the 24th to 48th hour with respect to the growth of the large chela. This point can be made clearer by simply renaming the periods

of irradiation with reference to the removal of each chela separately. Table 9 shows exactly the same data as table 8, rearranged in this way.

Table 9 shows that the time of greatest injury to the side of the large chela is between the 24th and 48th hour. However, since no injury was produced by irradiation from the 12th to the 36th hour, the effect observed between 24 and 48 hours must have been produced after the 36th hour. This fixes the susceptible period of the large chela between 36 and 48 hours after its removal, in the same way that the susceptible period for the small chela was fixed between 60 and 72 hours. If for convenience we compare the midpoints of these two periods, it is seen that the snap-claw requires only about 42 hours to reach a certain stage of development, whereas the

TABLE 9—*Effect of irradiation at various times on the regenerative ability of the two sides. (Period of irradiation named in relation to the removal of the chela in question.)*

Period of irradiation	Per cent of chelæ regenerated	
	At site of original pinch-claw	At site of original snap-claw
0 to 24 hrs. after chela removal....	100	97
12 to 36 hrs. after chela removal....	...	100
24 to 48 hrs. after chela removal....	98	60
48 to 72 hrs. after chela removal....	73	85
60 to 84 hrs. after chela removal....	61	...
72 to 96 hrs. after chela removal....	92	84
96 to 120 hrs. after chela removal...	93	84

pinch-claw needs about 66 hours to arrive at the same point. This once more emphasizes the fact brought out clearly last year, that the pinch-claw must have a start in growth in order to be able to compete with the snap-claw. What has been added to this concept is the approximate location of the moment at which the competition ends. Whereas the work of last year showed that with reference to a single individual *Crangon*, the critical difference in the time of start could be adjusted to within half an hour, it can now be said that that half-hour falls within a definite 12-hour period.

That it is the differentiating chela that is most affected by irradiation is brought out by the following experiment. Animals were irradiated between the 48th and 72d hours after pinch-claw removal; but in one set the snap-claw was removed 24 hours after the pinch-claw, while in the other set it was removed 48 hours after. Table 10 gives the results.

In this experiment, the small chela was being irradiated between the 48th and 72d hour after removal in both cases. The large difference in regeneration of the pinch-claw (73 *vs.* 96 per cent) results from the fact that when the chelæ are taken off 48 hours apart, there will be a certain amount of

reversal and some of the pinch-claws will be regenerating snap-claws; while when chelæ are removed only 24 hours apart, there will be no reversals and consequently none of the small chelæ will be coming back as large. In the latter case there was practically no injury from the irradiation, and 96 per cent of the animals regenerated a chela on the pinch-claw side. As to the large chelæ, in the 48-hour group irradiation followed immediately after snap-claw removal, thus preceding differentiation, and produced no injury. In the 24-hour group, irradiation took place between the 24th and 48th hour of snap-claw growth. Since conditions precluded reversal, the snap-claws were all returning in their original positions. They were correspondingly susceptible to injury, as only 68 per cent succeeded in regenerating any chela at all on that side.

TABLE 10—*Effect of irradiation applied from 48th to 72d hour after pinch-claw removal, on animals with chelæ removed 24 and 48 hours apart.*
(24.32 mg. Ra, at 2 cm. distance, for 24 hours.)

Time of snap-claw removal	Per cent of chelæ regenerated	
	At site of original pinch-claw	At site of original snap-claw
24 hrs. after pinch-claw.....	96	68
48 hrs. after pinch-claw.....	73	97

Another sign of the suppression of large chela formation by irradiation was found in the animals which regenerated both chelæ alike. Out of 206 animals irradiated, 18 produced equal chelæ. Of these, 17 had pinch-claws, and one had imperfectly differentiated snap-claws. In contrast, out of 126 non-irradiated animals kept at the same (room) temperature, 9 had both claws alike—5 small and 4 large. These figures, in addition to the foregoing material, definitely show the suppression of snap-claw formation by gamma rays. This point will again be referred to in connection with the influence of temperature on differentiation.

EFFECT OF TEMPERATURE ON THE PROCESS OF DIFFERENTIATION

The aim of the next series of experiments was to find out whether the length of the start necessary to insure reversal was markedly affected by temperature or not. The usual procedure of removing the pinch-claw first, and the snap-claw at stated intervals afterward, was followed at several temperature levels. The results are shown in table 11, and indicate quite a large thermal effect.

For instance, at 22° there were no reversals at all after an interval of 30 hours; but at 27°, 20 per cent reversed; at 32.5°, 32 per cent reversed; and at 35°, 47 per cent reversed. It is obvious, therefore, that the time taken to reach a state of equality of the two sides is markedly influenced. This shows that the attempt to balance both sides by allowing a definite

TABLE 11—*Percentage of chela reversal at various temperatures.*

Hours between pinch-claw and snap-claw removal	22°	27°	32.5°	35°
28	0	0	22	32
30	0	20	32	47
36	0	56	64	61
40	0	48	62	72
60	..	90	100	..
74	..	100	100	..
80	..	100	100	..

interval of time between successive chela removals must be done with reference to temperature. By increasing the numbers of animals used, and lengthening the intervals below 27°, it should be possible to work out the entire curve of 50 per cent reversal. This will furnish little indication of the character of substance "B," but will indicate the effect of temperature on the regenerative growth rate of the chelæ, up to the stage at which differentiation takes place.

TABLE 12—*Frequency of the two types of equal chelæ at various temperatures.*

Temperature	Total No. of animals with equal chelæ	Per cent having two snap-claws	Per cent having two pinch-claws
° C.			
22	10	10	90
27	18	33	67
32.5	9	44	56
35	14	79	21

It was shown previously that irradiation retarded growth, as indicated by percentage reversal. It is now seen that temperature has a similar effect. Researches are now in progress to compare these two effects and determine the thermal equivalent, in a biological sense, of a given amount of irradiation by gamma rays. Another feature of interest was the difference in production of snap-claws at different temperatures, as shown in table 12.

Parallel to the obvious increase in frequency of large chela production at higher temperatures was the better differentiation of snap-claws at higher

temperatures,[1] and the frequency of poorly differentiated snap-claws at the lower temperatures. None of the pairs of small chelæ tabulated above was due to lack of time for growth of a normal set of chelæ. Again the reduction in the number of pairs of large chelæ by low temperature is similar to the effect produced by irradiation over long periods. The simplest and most logical deduction is that the production of substance B, the hormone responsible for snap-claw formation, is enhanced by high temperature; and that this material is produced in such small quantities at lower temperatures or under irradiation (judging by the frequent occurrence of poorly differentiated chelæ) that there is not enough to form properly differentiated snap-claws.

In connection with this effect of temperature on differentiation, it is interesting to note that Morgan (1924), after removing the two large chelæ from some young fiddler crabs, reported their failure in the course of four molts to grow back anything more than small chelæ. He interpreted this as the inability of the organism, after once losing these large chelæ, to grow them back at all. This interpretation, however, is not compatible with his statement made in 1919, that a lost fiddle was promptly regenerated. The latter has been my experience with fiddler crabs. The difficulty with Morgan's young specimens was a time effect, possibly accentuated by low temperature. The following histories of cases of *Crangon* with two pinch-claws may help to elucidate this view.

TABLE 13—*Number of molts required for the development of a snap-claw in specimens of Crangon armillatus with two pinch-claws.*

Number of molts.....	1	2	3	4
Number of cases.....	18	3	1	3

HETEROGONIC GROWTH IN RELATION TO CHELA REVERSAL

Huxley's (1932) theory of heterogonic growth has recently been applied by Dawes (1934) to the subject of chela reversal in the Alpheidæ. Dawes does not report any experiments comparable to mine, but confines his attention to the mathematical analysis of chela measurements. There are several points in his interpretation on which we agree, but there are also some fundamental differences, which I propose to discuss here.

By a series of comparative measurements, Dawes shows that the snap-claw in the female is less modified than the same chela in the male. This merely substantiates Wilson's (1903) observations, which were made in descriptive rather than mathematical terms. We are in complete agree-

[1] These observations suggest that the differentiation of the male chelæ in *Inachus* during the breeding season, which occurs during the summer, may be associated with the higher temperature of the water at that time of year.

ment that there is no necessity for the old concept of an inhibitory substance, produced by the large chela, which prevents the small chela from developing into a snap-claw. I showed that this concept was untenable by inducing the growth of two snap-claws in animals that had previously been asymmetrical.

Dawes claims that a higher growth rate on one side of the animal than the other brings about an unequal distribution of the substrates for growth and causes one side to develop a snap-claw, the other a pinch-claw. In other words, the growth rate alone is responsible for differentiation. This explanation is difficult to accept, for several reasons. (1) In the classical experiments on localization, the independence of growth and differentiation was clearly brought out. Moreover, in *Crangon* a small but otherwise perfectly formed snap-claw may be only one-third the size of a newly regenerated pinch-claw. In extreme cases, I have had animals that produced a rudimentary snap-claw, barely visible to the unaided eye, while the pinch-claw was of normal size. In these cases, the greater size increase had been on the pinch-claw side. The growth rate, as I have shown experimentally, determines which side is to differentiate by enabling one side to reach a critical stage in development before the other; but it does not *produce* differentiation of the faster-growing chela. (2) Dawes claims that in older specimens, the pinch-claw normally grows faster, relative to the carapace, than the snap-claw. Why under these conditions the pinch-claw does not differentiate into a snap-claw he does not explain. (3) When only a pinch-claw is removed, the animal at the next molt grows a new pinch-claw. Dawes shows that under these conditions, the snap-claw decreases in size. In other words, the snap-claw has no positive growth rate, while the regenerating pinch-claw is growing very fast; yet such a pinch-claw never differentiates into a snap-claw. This should occur if a higher growth rate as such were the cause of differentiation.

There is one categorical statement in Dawes' paper that is not supported by data, namely, that the amount of growth is not correlated with the number of days available for regeneration. As my foregoing experiments have demonstrated that there are distinct differences in the growth of chelæ taken off at various times before molting, this statement must be denied, especially in the absence of the data on which it was presumably based.

In another place Dawes writes, "As was mentioned above, growth intensity beyond the lower limit of the size range studied must have been greater in the crusher than in the nipper to yield the size superiority of the former over the latter, so that this slackening of growth with increase in body size is no doubt as real as it is apparent." It does not appear to have struck Dawes as strange that his collections should have been made always after the high growth rate of the snap-claw had ceased to exert itself. Two methods of testing his assumption were open—to find the early stages with

the high growth rates, or to question the mathematical analysis of the data —as there is no possibility of questioning the huge size differences between the two chelæ.

The number of cases on which Dawes' calculations are based seem in several places too small, and the numerical data of too great variability, to permit some of the conclusions drawn. For instance, on page 554, two classes of carapace widths are compared with various other measurements as follows:

	Class	
	VI	VII
No. of cases	16	8
Carapace length	7.51	8.35
Crusher total bulk	95.01	119.1
Nipper total bulk	17.95	17.43

While in passing from class VI to VII the size of the carapace and the crusher (=snap-claw) increased, the size of the nipper (=pinch-claw) decreased. Perhaps this inconsistency is due to insufficient numbers of cases; nevertheless such is the material from which the deductions are drawn.

There is to my mind a real difficulty in the mathematical treatment of figures obtained from two morphologically different structures without regard to their qualitatively different origins. It will be an advance when qualitative differences can have corresponding numerical values assigned to them; but to ignore such differences merely because they are not susceptible of mathematical expression leads to grave error.

DISCUSSION

The secondary sexual modifications of chelæ in the Crustacea vary considerably, but can be arranged in a linear series with reference to extent. First come the unmodified forms in which the male and female are alike in possessing simple biramous appendages covered with setæ, similar to the ancestral type. This must be considered from the evolutionary point of view as the basic pattern, as it is the type commonly encountered in immature stages of development. Then comes the situation such as is found in *Inachus*, where chela modification develops only during the breeding season, and then only in the male; the chelæ of the female are never modified. Then there are cases like *Uca*, the fiddler crab, in which the female is again unmodified, but the male strongly and permanently modified, irrespective of the breeding season. Next come animals like the Alpheidæ, in which both sexes have one chela permanently modified, the other still covered with setæ and resembling the simple biramous appendage; but even here the modifica-

tion of the large chela is more marked in the male than in the female. Lastly there are forms which have both chelæ modified in both sexes. In these, however, the two chelæ are not modified equally. For instance, in the blue crab, *Callinectes sapidus* (Rathbun), the teeth of the chelæ are fine on one side and very coarse on the other.

The extent of possible change in these secondary sexual characters, the differentiated chelæ, was shown by Giard (1887) and Geoffrey Smith (1910-12). These authors observed that parasitization of the males of certain Crustacea causes a regression to the female form. Smith in particular, working on *Inachus*, showed that the parasite *Sacculina* does not alter the female chelæ but does change male chelæ to the female type. Potts (1906), however, had already placed this kind of alteration in its proper perspective when he pointed out that the regression of the parasitized male hermit crab was not so much to the female as to the primitive developmental type.

That some testicular hormone is involved in the differentiation of chelæ was indicated by Smith's discovery that the testes in some parasitized males were so altered that the cells resembled oögonia rather than spermatogonia. This concept is further supported by the work of Shaw (1928), who found in another species of *Inachus* that the male chelæ vary considerably as to the degree of differentiation, and that they regress to the female type after the breeding season. It is unfortunate that the female type of chela should also be the ancestral type, because the confusion of the two has obscured the nature of the developmental procedure. I have recently shown that chela modification in the fiddler crab is separable from the other sexual modifications involving the abdomen.

The periodic appearance of secondary sexual characters during the breeding season has been noted in many other animals, as in birds, deer, etc. Conversely, the disappearance of secondary sexual characters following the removal of the testes is known in a great variety of forms. I suggest that in the Crustacea we have an analogous condition. The evidence from parasitization and seasonal changes, as well as the familiar cases of sexual dimorphism, gives every reason to think that we are dealing with a sex hormone; and I suggest for further simplicity of writing to call this hormone "chelin." This name is to replace the term "substance B," as used in this and a prior publication for a chemical entity of unknown nature.

That such chemical entities do exist was shown by the recent work of Ruzicka *et al.* (1934). He was able to produce comb growth in capons by the administration of a pure synthetic substance, 3-epi-oxy-etio-allocholanon. There is no doubt that the comb in fowls is a secondary sexual character, and the enlargement which followed the injections of synthetic hormone was well outside the range of experimental error. The final test was that these combs regressed to their original size after the injections were discontinued. Here then we have evidence of the exact chemical nature of the testicular hormone which Butenandt (1934) has called "androsteron."

It is the convergence of all these lines of evidence that makes me suggest chelin as being responsible in the Crustacea for the modification of chelæ.

There is some slight evidence for the presence of a specific chemical entity in *Crangon*. The alcohol-soluble fraction of *Crangon* blood, taken at the time when snap-claw differentiation was found to occur, showed an absorption band in the ultra violet at 2550 Å, while a similar preparation from blood taken just after molting showed no such specific absorption in that region. Further investigation of this point is in progress.

So far I have dealt only with the substance which modifies chelæ, and have said nothing of substance "A" (Darby, 1934). This hypothetical substance I consider a basic chela-forming material, common to all Crustacea with biramous appendages. In connection with the larval development of *Uca*, Hyman (1920, 1922) describes a spoon-like biramous appendage which becomes the chela of the adult. Very little further modification of this appendage occurs after the last megalops stage, either in females or in the small chela of males. This unmodified biramous appendage closely resembles the pinch-claw in *Crangon*, and occurs in many other Crustacea as well. It is differentiated at a certain stage in larval development from a uniramous homolog, and I see no reason why it should not be attributed to the action of a definite chemical entity. The recent studies on organizers and the artificial induction of nerve cords by definite chemical substances must lead to the recognition of this possibility.

To study this phase of development, it will be necessary to experiment with the larval stages. There should be no difficulty in envisaging a system, starting with the gene in the chromosomal meshwork, which acts under one series of conditions (internal environments) to produce one result, and later, under other conditions, to produce a somewhat different structure. The potential capacity of the gene has too often been conceived as limited to the single factors which can be investigated by present methods of experimentation.

Biologists have frequently expressed bewilderment at the implications of a multiplicity of specific substances, one for each organ. Two answers may be made to this objection. First, that there must be an array of different substances is already implied in the number of genes found in even a single chromosome. Second, further knowledge of these substances may well lead to their classification, with consequent order instead of chaos. This process has repeated itself time and time again in the history of science.

Another phase of this work will deal with the manner in which special localized areas take up the substances which are being circulated in the blood stream. Such specificity of selection is well known in many instances, but little understood. That a part can be ready and capable of functioning without actually doing so until the proper stimulant is supplied has been brought out in a most striking manner by recent studies on hormones. The reversal of chelæ in the Alpheidæ is simply another example of this mechanism.

SUMMARY

Studies on regeneration of chelæ in *Crangon armillatus* (Milne-Edw.) have been continued, with the following results:

(1) The animal has a period just previous to molting during which only simple small chelæ (*i.e.* pinch-claws) are produced. This shows that the production of the chemical entity responsible for snap-claw formation is discontinuous.

(2) The differentiation of snap-claw takes place within a particular 12-hour period. The location of this period for each chela has been determined, and shows that the pinch-claw requires a longer time than the snap-claw to reach this critical point in development.

(3) Irradiation with gamma rays from radium has three effects: (a) It retards development; (b) when applied at the time differentiation is taking place, it damages the chela which is becoming a snap-claw; (c) it decreases the ability of the animal to produce a snap-claw at all.

(4) Temperature has a marked effect on the growth rate of regenerating chelæ, as judged by the length of the interval between chela removals necessary to control reversal.

(5) The production of snap-claws is enhanced by high temperatures and depressed by low temperatures.

(6) Various degrees of secondary sexual modification of the chelæ are shown to exist in the Crustacea. A hormone is suggested as being responsible for these modifications, to which the name "chelin" is applied.

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PAPERS FROM TORTUGAS LABORATORY, VOL. XXIX

VII

ANALYSIS OF THE CONSTANT DIFFERENTIAL GROWTH RATIO

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With five text-figures

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ANALYSIS OF THE CONSTANT DIFFERENTIAL GROWTH RATIO

Theories of growth are beset by the devil in the law of scientific parsimony on one hand and the deep sea of oversimplification on the other. The growth of a living organism (y) with time (x) or the relative growth of a part (y) with respect to the whole organism (x) may, in many cases, be expressed by the formula,

$$y=bx^k, \qquad (1)$$

wherein b and k are constants. The curve from this general equation is a parabola when k is positive and an hyperbola when k is negative. This is a simple relation which may be examined to determine how much information may be lost through oversimplification.

I

Schmalhausen [1] has used this equation to represent growth with respect to time. Huxley [2] and Teissier [3] have successfully fitted the equation to the growth of parts of an organism with respect to the whole organism and recently Needham [4] has extended the use of the equation to the chemical changes occurring as the organism becomes larger. The English workers adopted Pezard's [5] terminology of isogonic growth when $k=1$ and heterogonic growth when $k\neq 1$; but Teissier prefers Champy's [6] terms harmonic and disharmonic. The constant b was used by Lapique [7] to compare the amount of cephalization of different mammals and the constant k has been christened the constant differential growth ratio by Huxley [8] when the equation is used to express the growth of the part with respect to the growth of the whole organism.

The above equation has been used for some time in growth studies. Surface areas and weights were compared as well as the relation between brain and body size of mammals by Dubois.[9] The growth curves of a number of plants and animals were fitted with one or more hyperbolas in a series of papers by Stefanowska.[10] Because of this fitability by the same equation, she concluded that the growth process was fundamentally the same in both plants and animals. The relative prenatal growth of man has been fitted with a 4th degree parabola.[11]

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[7] L. Lapique, Bull. Soc. Anthrop., 5th ser., vol. 8, 248-270, 1907, Paris.
[8] J. S. Huxley, Nature, vol. 114, 895-896, 1924.
[9] E. Dubois, Bull. Soc. Anthrop., 4th ser., vol. 8, 337-376, 1897, Paris.
[10] M. Stefanowska, Compt. Rend. Acad. Sci., vol. 136, 1090-1093, 1903, Paris.
[11] C. M. Jackson, Amer. Jour. Anat., vol. 9, 118-166, 1909.

The same equation has been found useful in the examination of form changes during growth, especially those of weight and length. Hecht [12] and van Oosten [13] have been concerned with the variation of b for fish when k was arbitrarily made equal to three, but Keys [14] has indicated that the variation in both b and k denotes change of form by growing fish. Similar study of other animals has been pursued by Brody [15] and many new measurements and a discussion of the literature may be found in recent books.[2,3,4]

II

The greatest usefulness of this equation seems to be its objective presentation of the relations between the growth of a part and the whole organism. The value of k is usually determined from a graph on which is plotted the logarithm of the size of the part on the ordinate against the logarithm of the size of the whole, or the whole less the size of the part, on the abscissa. When the equation is applicable, k is the slope of the resulting straight line because equation (1) then becomes

$$\log y = k \log x + \log b. \qquad (2)$$

The value of the intercept b, which is the value of y when x=1, is not used often by modern investigators although its comparative value was recognized long ago.[7,9] The main advantage of the equation is that the corresponding sizes are directly related, thereby neglecting the time variable in growth.

The use of k has been criticized because the value obtained from the direct fitting of the sum of the parts would be different from fitting the several parts and combining the resulting k's.[2] The value k lacks dimensions and is a pure number.[4] Further the value of k shows, in some cases, breaks and discontinuities.[4,3] Small differences in rates and method of growth, though significant, are swallowed up in the general trend.[16] Bernstein's [17] criticisms from the mathematical viewpoint will be considered later.

The dimensionless number k may be considered to be a coefficient of correlation, but the usual linear correlation method is not applicable when working with the straight line form (2). The squares of the logarithms would have to be minimized by the method of least squares, which would involve considerable error when the variables covered much range in size. Nevertheless Schmalhausen [1] has published a least squares solution for ob-

[12] S. Hecht, Jour. Morph., vol. 27, 379-400, 1916.
[13] J. van. Oosten, Bull. U. S. Bureau of Fisheries, vol. 44, 265-428, 1928.
[14] A. B. Keys, Proc. Nat. Acad. Sci., vol. 14, 922-925, 1928, Washington.
[15] S. Brody, Missouri Agri. Exper. Station Research Bull. No. 103, 40 pp., 1927, Columbia, Mo.
[16] C. D. Davenport, Symposium on Quant. Biol., vol. 2, 203-208, 1934, Cold Spring Harbor, N. Y.
[17] F. Bernstein, Symposium on Quant. Biol., vol. 2, 209-217, 1934, Cold Spring Harbor, N. Y.

taining k. He justifies his solution in that the error from minimizing the squares of the logarithms of the variables may be less than the error of the measurements themselves.

Gray's [18] caution to the biologists about the danger of logarithmic representation is still pertinent. The diameter of the dot on the graph, unless the scales are proportionally large, may cover more range than the error of measurement of the variable, and when the dots are close together, or to a line, they may apparently fit the line but yet actually depart significantly from it. The use of numerical methods rather than the graphical procedures would seem to be indicated whenever the scales are not large enough to show clearly any small but significant variation. Great caution is required when averages are used to make certain that natural variation is not overminimized. It is surprising that in the rapidly increasing literature on heterogonic growth, one does not find the fits of the equation tested by any of the well-known methods for this purpose.

III

To illustrate the last section, the wet weights of the larger chela and of the body without the chela of some male hermit crabs, *Cœnobita clypeatus* Herbst, are plotted in figure 1A. These animals were carefully weighed at the Tortugas Laboratory during the summer of 1934. The chela was separated from the body by grasping it firmly in a pair of forceps and the crab then snapped it off at the breaking joint. This insured that the chelæ were comparable in size from the several animals. Considerable variation is shown because the actual measurements are used rather than arbitrary size averages.

The same data may also be plotted by means of the logarithm of the chela weight on the ordinate and the logarithm of the weight of the rest of the body on the abscissa. A line is drawn through these points to give a good fit, and the value of k is obtained as the slope of this line. The three-quarters of the measurements including the larger animals are given in the insert graph figure 1B. The part of the observations not included (25 per cent) is just as large when plotted logarithmically as that given in the figure. Consequently the variations of the smaller crabs are magnified and those of the larger crabs reduced by the logarithmic plot. The straight line best fitting all of the data is not the best for the larger 75 per cent of the animals shown in figure 1B, as its slope is a little too great. When this same line is drawn for the same equation on a non-logarithmic grit (fig. IA), the line (A′) fails to fit the data, and for the larger crabs the fit becomes progressively worse. One alternative is to assume that there is a break and that two straight lines should be used, one to fit the data for the smaller animals and another for the larger animals (line B′, fig. 1B) thereby getting

[18] J. Gray, Brit. Jour. Exper. Biol., vol. 6, 248-274, 1929.

good fits for both. Such a procedure would be justified only when there was a biological basis instead of arbitrarily locating the place of the supposed break.[19]

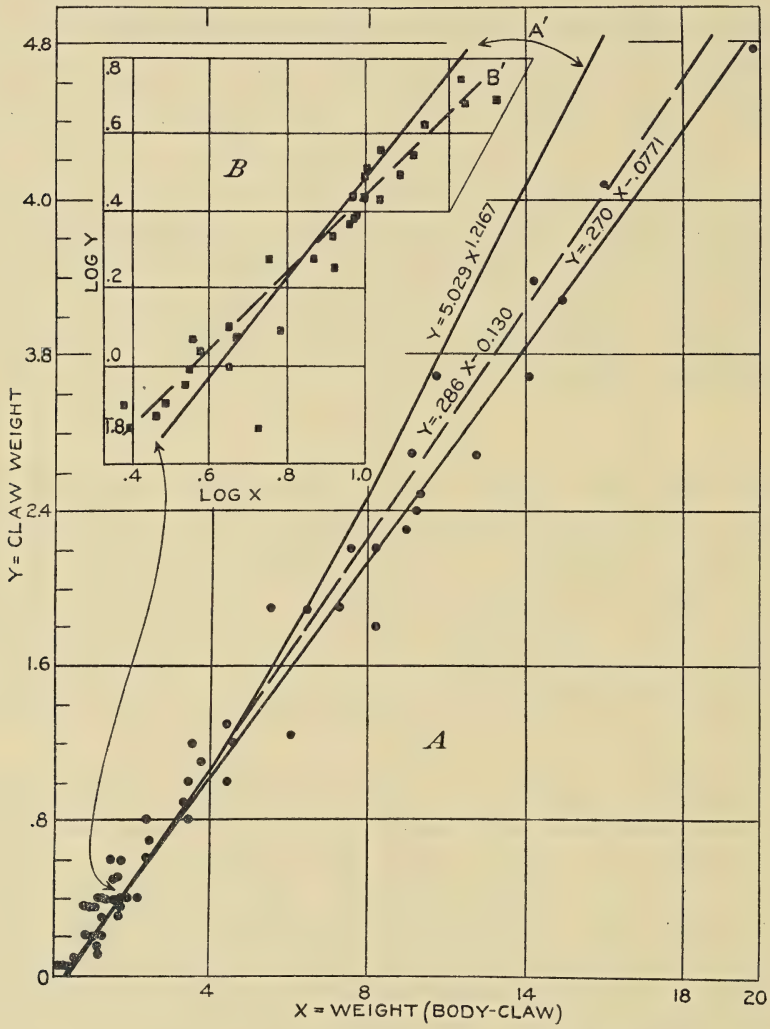

FIG. 1—A, linear plot of claw and body weight of the hermit crab. (Right hand line is C', center line is D'). B, upper part of A plotted logarithmically.

The data on the non-logarithmic plot (fig. 1A) show a continuous increase in size and no justification of a break at the point taken for the beginning of the insert graph. The data might be fitted by a straight line using the method of least squares which gives y=0.270 x —0.077, and this line is

[19] Cf. the criticism of Brody by MacDowell, E. C., E. Allen & C. G. MacDowell, Jour. Gen. Physiol., vol. 11, 57-71, 1927.

shown in figure 1A as line C′. In this case k does not appear as it is unity. This line, C′, fits the data better than the parabolic curve, but we may inquire whether another method of fitting may be even better.

The correlation method is appropriate because the data are linear and the solution will give the prediction equations or regressions rather than merely the best (least squares sense) fitting single line. The correlation is high, as r=0.96 with a standard error of 0.01. Because the correlation is not perfect or unity, there will be two different regression equations. One will give the best value of the chela weight for a given body weight, and this line is shown as line D′ on figure 1A, and the other will give the best value of the body weight for a given chela weight, both in terms of the variation actually found in this set of measurements. The standard error of the prediction values from line D′ is only 0.4 gram. The other regression line is not drawn, but it would have slightly more slope than line D′. Even without numerical test of fitting, line D′ appears to be the best fit, and the two regressions from the correlation method emphasizes that without perfect correlation there can be no single best line when we are interested in the growth relations.

The growth of the large chela of these male hermit crabs is isogonic with the growth of the body. Had the relation been heterogonic, the correlation method would still have been applicable, but a method for non-linear data would be necessary. There is precedent for the use of correlation methods in the study of growth [20] and they should receive more attention.[20a]

IV

The derivations of the equation (1) by Huxley [2] and by Tessier [3] both show that k is not an unique constant, but is the ratio of two or more different proportionality constants. As Huxley derives the equation $k=\beta/\alpha$, β being the relative growth of the part (dy/ydt) and α is the relative growth of the whole (dx/xdt). This shows that k can be a constant only when α and β are both constant, changing at proportionate rates or when they change reciprocally. The actual constancy of k may be examined only when the time of each measurement x and y is known and when the measurements are dimensionally comparable [16] by computing the respective rates. This has not been done because few available measurements of growth are adequate for the purpose. When the component factors in the ratio k do change, we are not justified in ignoring the time component of the growth measurements inherent in the use of the equation. The measurements themselves were ordered in time as the organism grew larger, even

[20] W. J. Crozier and S. Hecht, Bull. U. S. Bureau of Fisheries, vol. 33, 141-147, 1913; J. A. Harris, Proc. Soc. Exper. Biol. & Med., vol. 18, 4-5, 1913; J. A. Harris, and H. S. Reed, Biol. Bull., vol. 40, 243-259, 1921; S. Wright, Genetics, vol. 17, 603-619, 1932.

[20a] I wish to thank Mr. R. B. Brown for making the computations used in this section of the paper.

though the correct time scale for the organism may not have been the clock time observed when the measurements were made.

Would comparable growth conditions of part and whole lead to equation (1)? Huxley derived the equation by equating a general, common growth factor (G) for the part and for the whole. When this was done and time eliminated, Bernstein [17] found that the maximum size of the part and the whole were terms in the equation and that the graph of the logarithmic plot of the equation would not be a straight line unless k=1. The part of the graph representing the later growth will be straighter than that representing the earlier growth because of the differences in the reciprocals as the maximum size is approached (*e.g. cf.* figs. 3A and 4 below). Bernstein showed that there can not be a constant partioning of materials between the part and the whole throughout the growth period. The dimensions involved determine the results, Bernstein states, because " . . . the standard of measurement of growth, for instance in the limbs, is incomparable with the standard of growth measurement used for the total growth." (p. 213.) This is another explanation of the breaks that appear when straight lines are forced to fit the log-log plots of the sizes of organisms. The importance of proper measuring scales and of knowing the proportionality relations between different scales has been stressed by the writer elsewhere.[21]

V

The data of Jackson [11] on the growth of the brain and of the human body before birth are plotted logarithmically in figure 2A. The slope of the resulting line, k, is found to be 0.95 from the plot, or 0.99 by numerical computation. The equation fits the data well, and this is one of the less common cases when k is nearly unity. The relative growth rates per month of the brain and of the body were computed separately as the differences between the successive Naperian logarithms of the respective values.[22] The graph of these (fig. 2B) shows that the relative growth rates decline at very nearly the same rate for both the brain and the body. Because the brain is a hollow tube, the weight does not change as much as its spatial configuration. The ratio $k=\beta/\alpha$ is given on figure 2C, and this ratio is nearly constant except for the deviation shown in the ninth month. As there are only three measurements included in this interval and many more in the other intervals, this departure may be merely fortuitous variation. The use of the parobolic formula is justified for these data. The constant k is one, thereby disappearing and leaving a linear relation.

[21] O. W. Richards, Symposia on Quant. Biol., vol. 2, 157-166, 1934, Cold Spring Harbor, N. Y.

[22] R. A. Fisher, *Statistical methods for research workers,* page 28, 4th ed., xiii+307, pages, 1932, Edinburgh.

Comparisons between the growth of different animals may be made with the aid of the constant k when the biological conditions are related reciprocally. The advantage of this use has been explained by Harrison.[23]

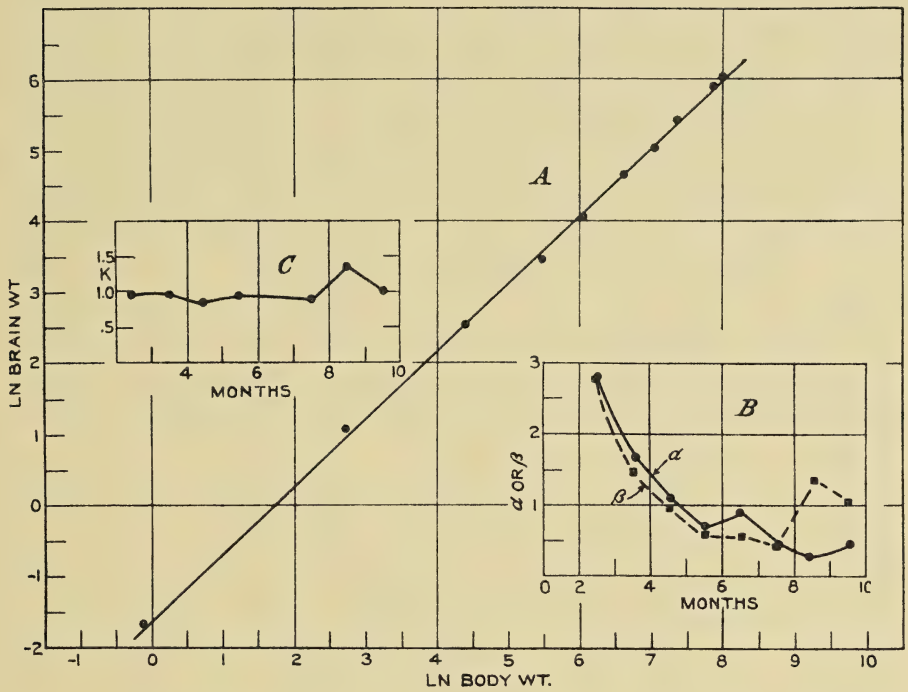

FIG. 2—A, logarithmic plot of brain and body weight before birth. Data from Jackson. B, relative rate of decreased growth of the brain (β) and of body (α) with time. C, change of $k=\beta/\alpha$ with time.

VI

The size of the eye of the larval salamander *Amblystoma tigrinum* logarithmically plotted against the size of the body [24] gives a nearly straight line (fig. 3A). With this large scale plot, deviations are noticeable and it is important to know whether these variations are fortuitous, represent breaks in the process, or result from the method used. The relative rates of the growth of the eye and of the body were computed and plotted in figure 3B, and the curves of the decline are both sigmoid. The ratio k is plotted in figure 3C, and instead of it being constant it is declining at a somewhat uniform rate, suggesting continuous process free from any breaks. The k obtained from the straight line form of the equation is entirely misleading and oversimplified.

[23] R. G. Harrison, *Heteroplastic grafting in embryology,* The Harvey Lectures, 1933-34, pages 116-157, 1935.

[24] V. C. Twitty, data communicated personally.

The measurement of the growth of an *A. punctatum* eye which has been transplanted upon an *A. tigrinum* shows that the decline of the relative growth rate of the transplanted eye is less than that of the normal *tigrinum* eye (figs. 3A, B). As a result of this difference, the values of k in time

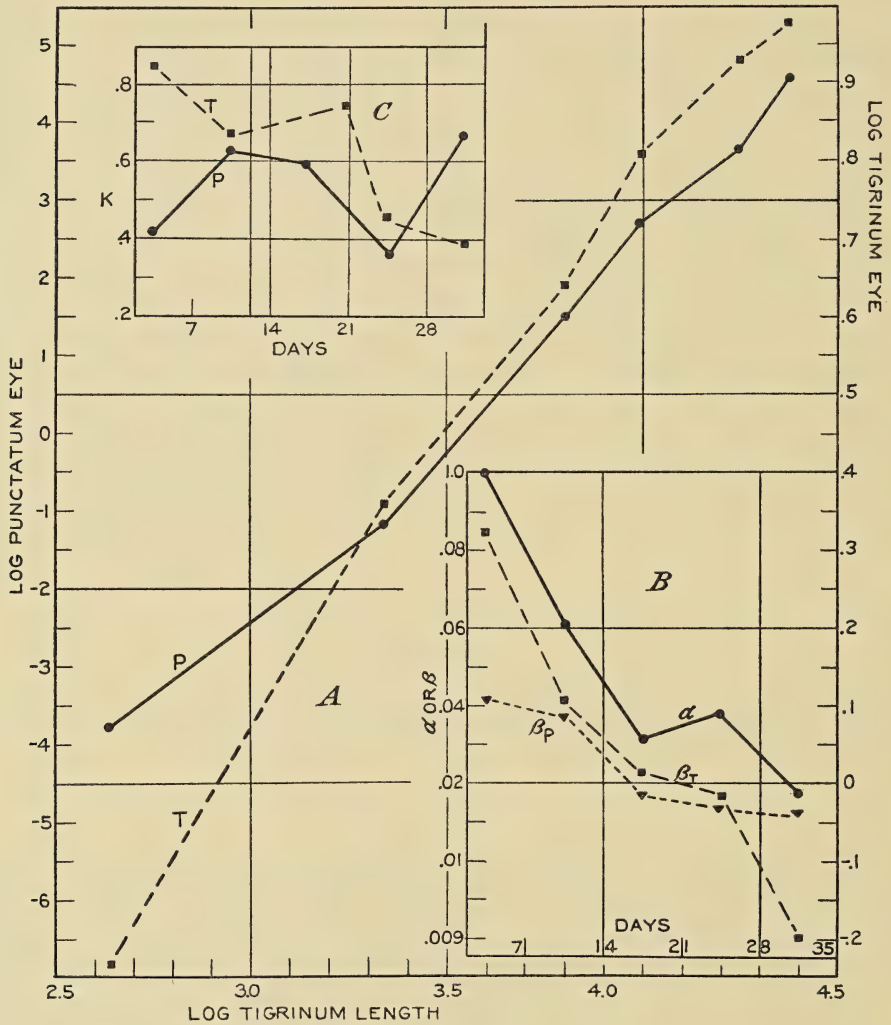

FIG. 3—A, logarithmic plots of the eye and body growth of *Amblystoma triginum* (T) and of the eye of *A. punctatum* (P). Data of Twitty. B, relative growth of the body length (α), the normal tigrinum eye (β_T) and of the transplanted *punctatum* eye onto *tigrinum* (β_P) with time. C, change in k for the above.

first increase (fig. 3C), then decrease and finally increase again. Both of these experiments show that k is not a constant and the use of the single average value of k, while giving a fair empirical fit to the equation, masks changes which occur as the animals grow. The resulting oversimplification prevents further analysis of the growth.

VII

A more interesting case of the systematic change of the heterogonic constant is to be found in the data of Moment [25] obtained by growing rats on an abundant and a reduced diet. The rats grew much more rapidly on the adequate diet, and the proportionate points were obtained by matching the animals on a basis of weight. The means of the measurements for the

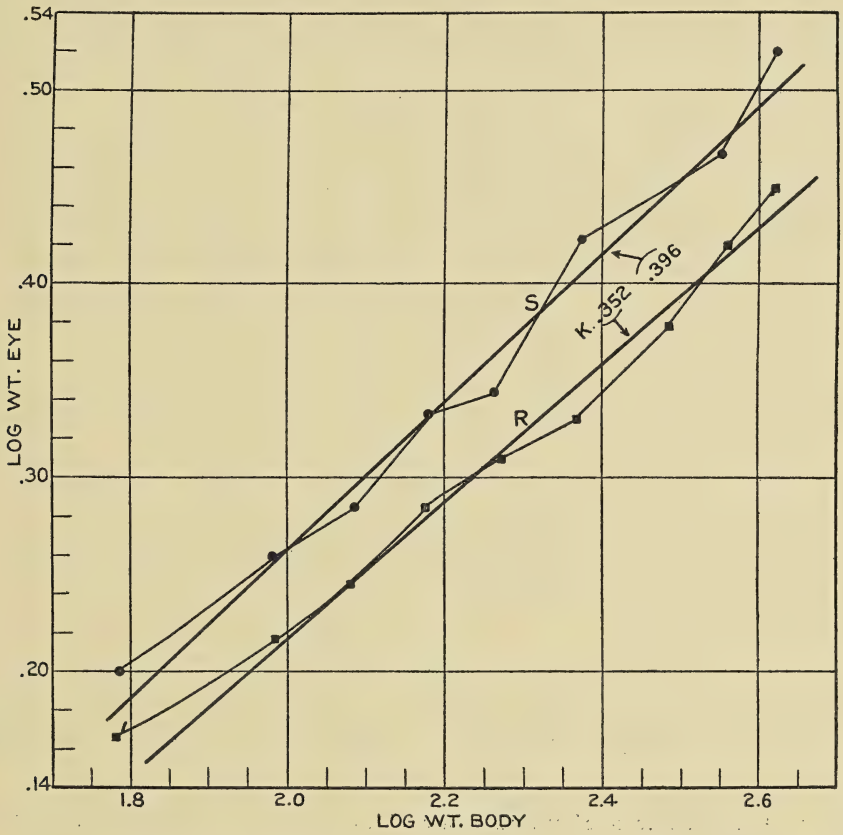

FIG. 4—Logarithmic plots of the eye and body size of rapidly growing (R) and slowly growing (S) rats. Data from Moment.

slow gaining and rapidly growing rats are plotted logarithmically in figure 4 and the best fitting line was drawn by eye through each set of points. The fit is fair except for the first and the last points, and from such a graph Moment concluded that the growth of the eye was heterogonic in both cases.

The relative rates of growth for the eye (β) and of the body (α) were computed in the same manner as before and they are plotted in figure 5. During the period of the experiments, the relative rate of growth of the

[25] G. B. Moment, Thesis, Yale University Library, 1932, and Jour. Exper. Zool. vol. 65, 359-393, 1933.

body declined, but with the rapidly growing rats there was a short period of increasing rate of growth before the decline set in. The eyes, on the contrary, show at first an increasing relative rate of growth for the rats with both diets. This period of increasing rate did not last as long when the rats were maintained at a high food level as with rats growing more slowly on a poorer ration. The graphs of k (fig. 5) show that k is not a constant but does vary. For the slowly growing rats this so-called constant passes through a maximum near the midpoint of the growth period. The maximum occurs earlier with the rapidly growing rats. The use of a single

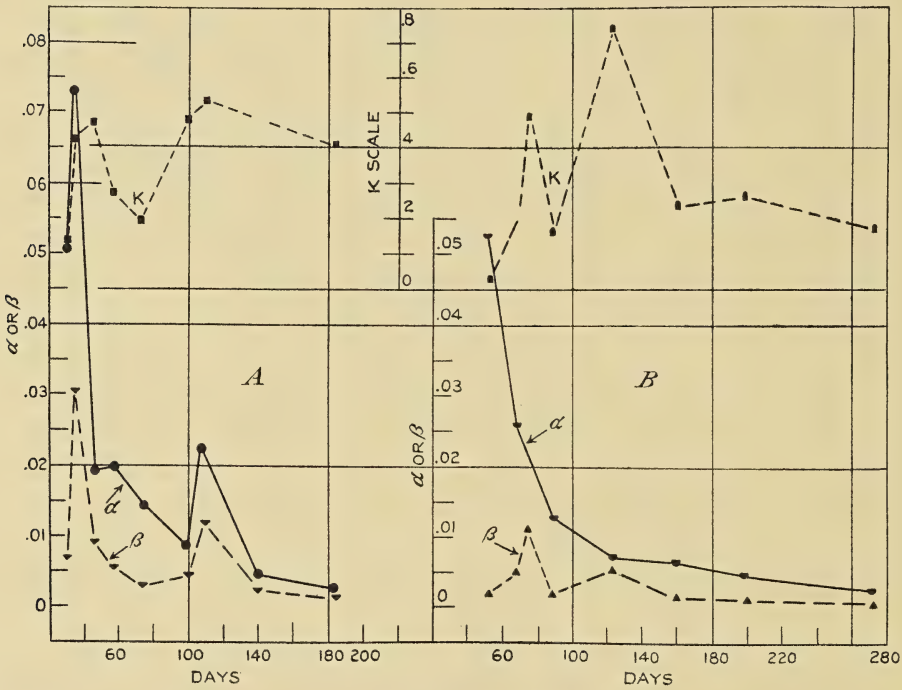

Fig. 5—A, relative rates of growth of eye (β), of body (α) and their ratio k for the rapidly growing rats. B, same for the slowly growing rats. Data for Moment.

average value of k from the logarithmic plot is an unjustified oversimplification obscuring the interesting changes in the growth rates of the rats which was, after all, the object of the experiment with the two different diets.

Moment, following the trend of other biologists,[26] suggests that his constant k is analogous to Nernst's partition coefficient and represents the balance of nutrition utilized by the eyes and by the rest of the body. The chemical partition coefficient is usually obtained with two or three chemicals in an otherwise homogeneous system which is very different from the exceed-

[26] *Cf.* Huxley[2] page 49, for discussion and references and W. E. Castle, Science, vol. 76, 259-260, 1932.

ingly heterogeneous system within the growing organism. The differences in the growth of the rats show when the analysis is made as shown in figure 5, but these differences are entirely obscured when the growth is examined only by means of the slopes of the two lines of figure 4. The comparison of k's from different organisms would be permissible only when it is known that the experimental conditions leading to the pure, dimensionless number k were similar and comparable (*cf.* also [23]). Thus the theoretical objections to a partition coefficient stated in section IV are borne out by the examination of an actual experiment.[27]

SUMMARY

A critical examination is made of the use of the heterogonic constant k in the formula $y=bx^k$ which may be used to fit the growth of a part (y) of an organism with respect to that of the rest of the organism (x). This value k is the ratio of two or more proportionality constants, and for experiments with growing rats and salamanders it is shown not to be constant but to vary during the growing period. Both theoretical and practical objections to the concept of a constant partition of materials between the part and the whole organism are advanced. For prediction equations the general correlation methods are superior to the above formula. This was illustrated by the linear relation between the size of the larger chela and the body of the male hermit crab *Cœnobita clypeatus*. The analysis of growth can not neglect the underlying reactions which may change with time, and the use of a single average value of k (or a constant partition coefficient) is, in many cases, an unjustified oversimplification which prevents further elucidation of the growth of the organism.

[27] Since this was written my attention has been called to a paper by J. S. Huxley, Nature, vol. 133, 982, 1934, which shows a progressive change in the constant k for the pistol-crab. Varying heterogonic constants are also reported by R. F. Blount, Jour. Exper. Zool. vol. 70, 131-185, 1935.

PAPERS FROM TORTUGAS LABORATORY, VOL. XXIX

VIII

STUDIES ON THE BIOLOGY OF TORTUGAS CORALS

I. Observations on Mæandra areolata Linn.

By C. M. Yonge

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With three plates and 1 text-figure

[Issued December 9, 1935.]

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STUDIES ON THE BIOLOGY OF TORTUGAS CORALS

I. Observations on Mæandra areolata Linn.

HABITAT

Mœandra areolata is one of the commonest corals of the Caribbean and Florida reefs. It is, as will be shown in this paper, a highly specialized species adapted for life in a restricted environment and it is somewhat surprising that so little is known about it. Vaughan (1919 *b*) states that "*M. areolata* is not a true reef coral. It thrives best on the flats behind the reefs or in water 10 to 12 fathoms deep off the reefs proper. As it has no firm basal attachment it can not resist the impact of the waves of rough seas." Verrill (1901) speaks of this species as being pedicellate when young, but usually becoming free when old. At the Tortugas it is very common in sheltered areas on the inner side of the circle of reefs where wide stretches of sand occur. The best collecting ground is the lee of Bird Key Reef. The summit of this reef, which is widely exposed at low-water spring tides, is bounded on the lee by a narrow belt of the delicate *Porites furcata,* beyond which extends a sandy area some 70 yards wide and ranging in depth from 6 inches to 3 feet at low-water springs. The sand is bound together to some extent with turtle grass. The only coral found normally in this region is *M. areolata* which is everywhere abundant, individual specimens being seldom more than about one foot apart. They have *no basal attachment whatsoever.* They can be picked up by hand at low water or by means of a long-handled net when the tide is in. They are round or oval in shape and stand out conspicuously against the background of white sand, owing to their medium or dark-brown color (due to contained zooxanthellæ). Examination revealed that, despite the soft nature of the substratum, the tissues are invariably completely exposed, only the underlying naked skeleton (which is frequently almost flat) lying beneath the surface. Wherever similar conditions prevailed, *M. areolata* was always the dominant and frequently the only species of coral. It is unquestionably highly adapted for life on a sandy bottom.

In a later paper in this series an account will be given of the changes which have taken place in the physical conditions, and as a result in the fauna of the moat at Fort Jefferson since this was first breached by the sea in 1919. Vaughan (1918) in an account of the coral fauna of the moat before this occurred mentions *M. areolata.* Careful examination of the bottom of the moat failed to reveal a single specimen of this coral in the summer of 1934. Free circulation of water being now impossible, the moat is a sediment trap and the bottom is covered with a fine slimy deposit. There is apparently, therefore, a limit to the softness of the substratum on

which *M. areolata* can maintain itself. This was confirmed by transplanting a number of individuals from the lee of Bird Key Reef to the bottom of the moat. At the end of two weeks (the experiment was unfortunately carried out toward the end of my visit), although all the animals were still visible, many showed unmistakable signs of sinking into the soft mud. The tissues were no longer everywhere raised above the bottom.

Observations carried out with the aid of the diving helmet in water up to about 3 fathoms indicated that *M. areolata* occurs at these, and presumably at greater, depths wherever there is a firm sandy bottom. It does not occur on mud and was never found attached in any part of the reef proper.

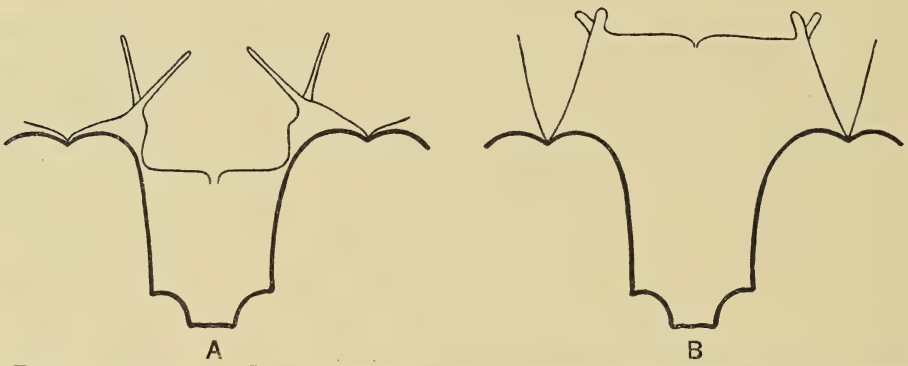

FIG. 1—*M. areolata.* Diagrams showing *A*, expansion for feeding; and *B*, expansion (*i.e.*, distension of the tissues with water) for cleansing. × 2½.

FEEDING REACTIONS

Vaughan (1913, 1919a) has given an account of the feeding of *M. areolata.* He states that not only are pieces of meat or living zooplankton organisms captured by the tentacles, but that the stimulus of animal matter causes a reversal of ciliary currents, food being carried in mucus streams to the mouth.

Vaughan's conclusions were confirmed so far as the tentacular feeding is concerned. If animal matter is presented to the tentacles of an expanded specimen it is seized securely. The tentacles then fold in over the disc as described by Carpenter (1910) for *Isophyllia* and by Yonge (1930) for allied genera such as *Favia, Cœloria, Hydnophora* and *Symphyllia.* The nature of the reaction and the expansion of the tissues associated with feeding are shown in figure 1, A.

The most careful examination failed to reveal any evidence of ciliary reversal. This was, incidentally, disappointing because I had hoped to do work on the conditions controlling reversal. When meat juice was placed on contracted specimens during the day time there was usually no expansion

of the tentacles but the mouths opened to their maximum extent, as much as 5 mm. long and 3 mm. wide. At the same time a great quantity of mucus was extruded from the body surface. Mucus strings were formed in which the fine particles of meat (to which carmine was usually added) were entangled and the whole was drawn into the mouth. On the other hand, when small pieces of meat were placed on contracted specimens, no matter in what region, they were invariably caught in ciliary currents and carried to the edge of the tissues. Carmine particles not mixed with meat juice invariably suffered the same fate.

When the tentacles were expanded at night, meat was readily taken by them, but carmine particles placed on the surface in the vicinity where food was being ingested were invariably removed by cilia. A mixture of meat juice and carmine caused the same reactions as in the contracted state.

The *apparent* reversal of ciliary currents is due undoubtedly to the excessive production of mucus and the consequent formation of mucus strings, which are caught by the ciliary tracts in the stomadæum which are exposed when the mouth is opened. These strings are raised above the surface to some extent (they can easily be picked off with a needle) and so do not come under the influence of the cilia covering the ectoderm. If carmine alone is applied, much less mucus is secreted, while the mouths do not open, and in consequence the carmine is rapidly carried away. The application of meat juice brings about an *unnatural reaction*. The normal food of the animal consists of zooplankton, probably exclusively (see Yonge, 1930), for which small pieces of meat are a satisfactory substitute. In these cases there is probably a local secretion of mucus which aids the transfer of the prey to the mouth and down the stomadæum. Parker (1919) regards mucus production as "a strictly local response to a local stimulus." When meat juice is added, this local reaction becomes general and instead of the mouth nearest to the food opening, all of those within range of the meat juice (usually all) open. The apparent reversal of cilia is therefore the result of quite abnormal conditions under which material is carried *over* the top of the ectodermal cilia. Vaughan states that after the coral is gorged with food, "ciliary reversal" no longer occurs, all particles being carried away. Here again there is an alternative explanation, namely that under these conditions the mouths no longer open in response to the stimulus of food and consequently the ingoing ciliary tracts are not exposed. It was also observed that after some time the excessive secretion of mucus ceased and particles were then caught in the ciliary currents and quickly removed. A statement by Carpenter (1910) is illuminating in this connection. Discussing the feeding reactions of *Isophyllia*, he states that carmine mixed with meat juice is usually carried to the peripheral margin of the polyp. He repeated this experiment many times with different polyps, "and occasionally succeeded in obtaining a reversal of the cilia, which caused the carmine

grains to move toward, and not away from, the mouth." He was, however, "unable to obtain consistent reactions, and never could predict what the result would be."

This was precisely my experience with *M. areolata*. Conditions are quite different in corals where the ciliary currents undoubtedly reverse, namely in many species of *Fungia* and in *Herpetolitha stricta, Döderleinia irregularis, Tridacophyllia lactuca* and *Merulina ampliata* (Yonge, 1930). In all of these the presence of animal matter causes an *invariable* reversal of ciliary currents, which return to the normal a definite time after the stimulating substance has been swallowed. There is never the slightest deviation from this procedure. It was possible to demonstrate that in *every case* reversal of ciliary currents was associated with the presence of *small tentacles* which could only sweep over a very limited portion of the tissues. This applied even to different species within a genus. The majority of species of *Fungia* have short widely scattered tentacles and in all of these reversal was found, but in one species, *Fungia actiniformis* var. *crassitentaculata*, the tentacles are large and extremely long (4.5 cm. in an animal with a maximum disc diameter of 7 cm.) and completely obscure the disc and the mouth. No reversal was ever found in this species.

I commented at that time (Yonge, 1930) on the difference between *Cœloria*, in which I found no evidence of ciliary reversal, and the closely allied genus, *Mœandra*, where Vaughan had reported its presence. I accounted for this difference at the time by stating that judging by Vaughan's photograph of expanded *M. areolata* (1919 *a*, pl. xvii) the tentacles of this species, unlike those of *Cœloria*, do not cover the entire surface. Personal examination of fully expanded specimens revealed, however, that such is not the case. Food on any part of the surface is within easy reach of the tentacles.

Vaughan also states (personal note in my paper, Yonge, 1930) that ciliary reversal occurs in *Manicina gyrosa*. He describes the tentacles of this species as being extraordinarily small, not reaching from the edge-zone to the mouth. I was unable to find any sign of ciliary reversal, obtaining exactly the same reactions as in *M. areolata*. The tentacles certainly do not expand well in captivity. The maximum expansion observed was about 2 to 3 mm., though the edge of the disc which bears them was occasionally seen raised to such an extent that the tissues between adjacent discs was completely obscured. During the Greater Barrier Reef Expedition where it was possible to examine corals in reef pools at night by means of a lantern and so to observe them when fully expanded in nature, I was forcibly impressed with the fact that few, if any, corals will expand fully in aquaria. I have no doubt that the tentacles of *Manicina gyrosa* are capable of great expansion in nature and that there is no necessity here for ciliary reversal, which never occurs without due cause.

CLEANSING MECHANISMS

A coral is a sedentary animal with a large surface. It has long been realized that survival is frequently dependent on the presence of efficient mechanisms for the removal of sediment. These are provided by the cilia which everywhere cover the surface. I have dealt in detail with the nature of the ciliary currents in a wide range of Pacific reef corals (Yonge, 1930) while Marshall and Orr (1931) have studied the general problem of the influence of sediment and of its removal in many of the same species. *M. areolata,* living as it invariably does on a sandy bottom, is in particular need of efficient means for the removal of sediment. During rough weather it will be subjected to a rain of fine particles of sand stirred up from the bottom.

A series of experiments were carried out in which *M. areolata* and the allied species *M. clivosa* and *M. strigosa* were placed in large glass jars containing some two and a half litres of sea-water and their surfaces covered evenly with three grades of sediment—mud, fine sand and coarse sand. In all of these animals, material was first removed from the disc by currents which carried it outward on to the cœnosarc covering the intervening ridges. There it was caught in currents which removed it from the surface of the colony.

Similar differences were observed between the rate of removal of all types of sediment by the three species. It will be sufficient, therefore, to describe the results obtained with the fine sand (which probably corresponds most closely with the type of sediment chiefly encountered in nature).

M. aerolata—Sand very rapidly removed, very little left at the end of 30 minutes. Disposition of sand causes the disc tissue to be raised above the level of the general surface thereby permitting the rapid transference of sand.

M. clivosa—Sand entirely removed in between two and three hours. Disc tissues expand, not to same extent as in *M. areolata,* but appreciably higher than intervening cœnosarc. Tissues of adjoining discs tend to overarch the ridge between and sand carried away in the groove so formed.

M. strigosa—Cleansing a little slower than in *M. clivosa,* the disc tissue seldom rising above the level of the intervening ridges.

All of these corals remove sediment rapidly from the surface. The differences between them can be correlated with the habitat of the species. *M. areolata* lives free embedded in sand, *M. clivosa* forms encrusting masses on the surface of boulders and usually possesses a wide flat surface on which sediment will readily collect and from which removal will not be aided by gravity. *M. strigosa* forms rounded colonies and the greater part of the sediment which falls upon it will tend to roll off.

The action of the cilia is aided, especially in *M. areolata* and *M. clivosa,* by a notable expansion of the tissues of the disc. This will be discussed a little later.

Marshall and Orr (1931) found that *Fungia,* as previously recognized by Wood-Jones (1912), is exceptional in its power to remove sediment. It can uncover itself when completely buried in sand, a fact forcibly demonstrated by a series of photographs which illustrate their paper. Now *M. areolata,* though belonging to a different family of corals, has a similar mode of life to that of the Fungiidæ in general (*Fungia, Herpetolitha, Döderleinia,* etc), both being unattached when fully grown and living on a sandy bottom. They are exposed to the same dangers and may be expected to have similar means of overcoming these.

A series of experiments were conducted in which *M. areolata* and a variety of other corals, including *M. clivosa* and *M. strigosa,* were completely buried in fine sand which covered the uppermost tissues by at least 2 mm. The *only* coral which was able to uncover itself (and which *invariably did so*) was *M. areolata.*

Exact records were made of the behavior of one specimen the skeleton of which was 11 cm. long and 5.5 cm. wide. These are summarized below.

After 50 min.—Strip of tissue 4 cm. long and 1 cm. at the widest part exposed. All *disc* tissue, flanked by short stumpy tentacles. Sand passing over sides of this.

After 1½ hours—About 5 cm. exposed, all comprising central disc.

After 2½ hours—About 6 cm. long and 2.5 cm. at widest. Still all disc tissue, greatly distended; mouths partially open and ridges in stomodæum exposed.

After 3 hours—Lateral discs beginning to uncover. Appearance of ridge of sand about 1 cm. beyond rim of exposed areas.

After 4½ hours—Now 8 cm. long, 4 cm. at widest where lateral disc tissues being exposed. Ridge of sand moving out and increasing in height.

After 5½ hours—Area 9 cm. long by 5 cm. at widest now exposed. Outer sides of disc tissue now anything up to 0.75 cm. about the surface of the sand which is quickly sliding away in steep sided grooves between discs.

After 6½ hours—Area 11 cm. by 7 cm. exposed (greater than that of skeleton owing to great distension of tissues). Sand being carried away rapidly in areas between discs.

After 8½ hours—Area 11.5 cm. by 7 cm. exposed. Disc tissues which hitherto have been horizontal now partially overhung by edge zone with tentacles expanded to about 5 mm. Of the fourteen lateral discs only the ends of seven now under the sand.

After 10½ hours—Entire surface now raised above surface of sand and fully dilated (area, 13×7.5 cm.). Maximum elevation above sand now 3 cm. Smooth, rounded surfaces of the distended discs entirely obscure intervening tissues and only interrupted by lines of tentacles which touch one another. Individual discs up to 2 cm. wide. Beyond outer edge of lateral discs there is a clear area about 1.5 cm. long extending down to attachment to skeleton. Sand level that of the naked skeleton.

On the following day a similar experiment was carried out with the same specimen and photographs, reproduced in Plate I, figures 1 to 6, were taken from time to time.

M. areolata has a remarkable power of uncovering itself when buried in sand by the combined agencies of ciliary action and of distension. The latter process consists of taking in water so that the tissues are literally blown out. In the specimen particularly studied the disc tissues were raised about 2 cm. above the skeleton. After the completion of the second experiment, the contained water was drained off and measured 113 cc., which represents taking into consideration the lateral distensions, a layer of water at least 2 cm. deep over the entire surface.

The mechanism of distension with water consists, clearly enough, of a combination of inwardly directed ciliary currents and a universal relaxation of the muscle bands in the mesenteries. Distention of this type is quite different from expansion for feeding, as is indicated by a comparison between figure 1, A and B. Feeding is impossible when an animal is distended for cleansing. Though the tentacles are distended to some extent like the other tissues, they do not react to food, and are incapable like the edge-zone of overarching the disc owing to their distended condition.

It is interesting to compare the reactions of *M. areolata* with those of *Fungia*. The latter succeed in uncovering themselves by the exclusive action of cilia (the absence of any distension of the tissues is clearly seen in the photographs of Marshall and Orr.) But in *Fungia* the tissues extend *under* as well as over the disc. The cilia on both surfaces beat outward, a fact which probably accounts for the ability of these animals to uncover themselves. In *M. areolata* the tissues extend only a short distance beyond the margin on the under side of the skeleton, and the cilia on this region beat downward. It is only as a result of the remarkable capacity for dilating the tissues with water that *M. areolata* is able to push its way upward through the sand and so permit the cilia to carry the sand away from its surface. The Fungids and *M. areolata* live under similar conditions, encounter the same danger of burial beneath shifting sand and are both able to overcome this danger, but they do so in different ways.

REPRODUCTION AND GROWTH

Regular collections of *M. areolata* were made during the seven weeks spent at the Tortugas and planulæ were obtained in great numbers over two periods. The first of these extended from July 7 to 13 and the second from August 2 to 9. Between these two periods no planulæ were obtained. As a result of observations carried out during the course of the Great Barrier Reef Expedition, Marshall and Stephenson (1933) have shown that *Pocillopora bulbosa* breeds discontinuously. During the summer and early winter (December to April in the southern hemisphere) planulæ were extruded about the time of new moon and during the winter months about

the time of full moon. During the summer of 1934 new moon fell on July 11 and August 10, *i.e.* about the end of the period of planulation in *M. areolata* in each month. The only previous record of planulation in this species at the Tortugas is given by Boschma (1929), who states that in 1925 he collected planulæ on July 19 and following days. In that year there was a new moon on July 20.

The limited data available indicates, therefore, that planulation in *M. areolata* during the summer months at the Tortugas has a lunar rhythm, culminating about the time of new moon.

Boschma (1929) has given an account of the post-larval development of *M. areolata.* The planulæ settle down in the usual manner on a hard surface. Knowledge appears to be totally lacking, however, as to the stage at which and the manner in which the animals become detached. There is no stalked individual as in the Fungiidæ, from which free individuals can be detached. In the hope of obtaining information on this point as large a collection as possible was made of young *M. areolata.*

Examination of these revealed that, after settling, the individual must grow upward and outward. There is formed in this way a flat-topped calyx, round or oval in outline, with a narrow basal attachment. The skeleton at this stage bears a striking resemblance to that of a stalked *Fungia.* The smallest specimen collected (Plate 2, fig. 7) illustrates this condition very well, being 14 mm. high, 3 mm. in width across the base and 13 mm. across the calyx. After attaining about this height, outward growth greatly exceeds upward growth, the next specimen (Plate 2, fig. 8) having a calyx 26 mm. long and 16 mm. wide, but being only 15 mm. high. The diameter of the basal attachment varies, but this is probably due to certain individuals settling in cracks and hollows to the sides of which the skeleton adheres during upward growth. An example of this condition is shown in Plate 2, figure 9, the diameter of the apparent basal attachment being about 12 mm. and that of the calyx (roughly circular) some 24 mm.

Subsequent growth leads to the formation of an oval or round skeleton. At the same time the tissues gradually recede from the under surface. This process is illustrated in Plate 2, figures 10 to 12, the maximum diameters of the calyces and edge-zone tissues respectively in these specimens being as follows: (fig. 10) 41 mm., 15 mm.; (fig. 11) 52 mm., 11 mm.; (fig. 12) 86 mm., 8 mm. Owing to this withdrawal of the tissues, the underlying portions of the skeleton are left unprotected. It is for this reason that the older specimens, always found detached, have frequently lost all traces of the basal attachment. Constant rubbing against the sandy substratum is probably the prime reason for this, though in some cases there are large cavities in the under part of the skeleton, due to the action of boring animals or plants. All the largest specimens (reaching a maximum diameter of over 12 cm.) are either flat on the under side (as shown in Plate 3, fig. 13) or else markedly concave. The latter condition is due sometimes to a curling

over of the upper portion, probably the result of environmental conditions while still attached.

It is impossible to say at what stage detachment occurs. Beyond doubt this will depend primarily on environmental conditions. If an attached individual is exposed to powerful wave action, it will be detached much sooner than another which is growing in a sheltered locality. It will also depend on the width of the basal attachment, which, as already shown, varies considerably. The largest attached specimen found had a maximum diameter across the calyx of about 70 mm., whereas the smallest unattached specimen had one of no more than 25 mm.

The work of Vaughan (1910, 1911, 1916) has provided extensive data on the growth-rate of *M. areolata*. He found a rapid rate of growth for the first two to four years, when a maximum diameter of some 60 mm. was attained, followed by a decline. His oldest specimens, 8 years and over in age, attained a maximum diameter of between 80 and 100 mm. In all cases the skeletons were cemented to tiles so that no data is available as to the age when detachment occurs.

It proved possible, by a comparison of skeletons of various sizes, to determine the manner in which later growth takes place. The young calyx, as already indicated, may be oval or round, but apparently it always becomes oval at a rather later stage, although the mature colony may be round (*e.g.* Plate 2, fig. 12). Elongation is accompanied by the formation of lateral valleys, which appear, when growth is unimpeded by mechanical obstacles, usually opposite one another. Upward growth is now more equal to outward growth, so that eventually a colony is formed, flat on the under side (except where the basal stalk persists) and rounded above.

Several individuals, representing planulæ which, as Boschma (1929) has shown, frequently settle down together, may fuse during development. Under these conditions the ultimate shape of the colony is *not* affected. This is well shown in Plate 3, figures 14 and 15. The two colonies figured are of about the same size and have the typical oval shape, but whereas the first has arisen from a single planula, the second has arisen from three. The number of individuals which compose any colony can always be determined by an examination of the valleys. If these are all in communication with one another (as in Plate 3, fig. 14), then the colony has arisen from a single planula; if, on the contrary, the valleys are divided into two or more self-contained series, then the colony has been formed by the fusion of a similar number of young individuals. The boundaries of the three individuals which have fused to form the single colony shown in Plate 3, figure 15, are indicated by broken lines.

This capacity for the formation of a colony with a smooth outline, no matter how many constituent individuals may have gone to its composition, is clearly of value to the animal because the chances of survival of a colony when buried under sand will be greater if the outline is smooth, *i.e.* the margin reduced to the minimum, than if it is irregular.

DISCUSSION

The results of this study of the habits and reactions of *M. areolata* draw attention to an aspect of corals which has in the past been almost completely overlooked. I refer to their power of adaptibility. The skeleton of the coral has much too frequently monopolized the attention of the worker to such an extent that he has completely failed to consider the living animal which forms it. But the Madreporaria are one of the most successful groups existing at the present time and, like all highly successful groups, they are eminently adaptable and so capable of exploiting every type of environment within the restrictions imposed (in the case of reef-building corals) by temperature, depth and salinity.

I have already had reason (Yonge, 1930) to discuss the many adaptations in their feeding mechanisms, of which the most conspicuous, the use of cilia in place of tentacles where these are small, has been referred to in this paper. Reef-building corals are undoubtedly specialized for securing zooplankton organisms of all sizes and in all situations.

Cleansing mechanisms are no less important than feeding mechanisms in sedentary animals, exposing such a wide surface on which sediment can collect. The removal of sediment, except in a few very specialized cases, is carried out by cilia. The primitive condition is probably represented by corals such as *Favia* in which there are large polyps with large tentacles concerned exclusively with food capture, the cilia which everywhere cover the cœnosarc being solely cleansing in their action. Corals of this type, as shown by Marshall and Orr (1931), cleanse themselves without any assistance from water movements.

From corals of this type, evolution would appear to have taken place in two directions. One of these has culminated in the appearance of corals with small polyps in which cilia assist feeding by carrying food particles up the column (as in *Porites*) and finally across the colony to the individual polyps as in the Agariciidæ in general. In one of these, the Indo-Pacific genus *Cœloseris*, the cilia always carry particles exclusively to the polyps, cleansing being entirely by wave action (Yonge, 1930). Actually in all small-polyped corals water movements materially assist cleansing and these corals are particularly common in shallow water and actually between tide marks. Many are encrusting forms.

The other line of evolution has seen the development of cilia to such an extent that the animals are capable of existing freely on the surface of sand and even uncovering themselves when buried beneath it. This is what has happened in the case of the Indo-Pacific Fungiidæ and it has enabled these animals to exploit a fresh habitat. Ciliary reversal is common in this family, but this, as already stated, is due to reduction in the size of the tentacles. *M. areolata* has arrived at the same end as the Fungiidæ but by a somewhat different route, the absence of tissue, and so of cilia, on the under side of the skeleton being compensated for by the remarkable power of distending the tissues.

Thus from the original large-polyped corals with a wide range have probably evolved small-polyped corals typical of shallow waters in which cilia assist in feeding, and water movements assist in cleansing, and also large-polyped detached corals which live on sand and have the cleansing mechanism correspondingly developed. It is worthy of note that in the single genus *Mœandra*, there are typical large-polyped individuals with massive rounded skeletons, such as *M. strigosa* and *M. labyrinthyformis*, others which form encrusting colonies such as *M. clivosa* (though cilia play no part in feeding here) and a detached species living on sand, namely *M. areolata*.

I wish to acknowledge my indebtedness to the Government Grant Committee of the Royal Society of London and to the Carnegie Institution of Washington for financial help which enabled me to visit the Tortugas Laboratory. I also wish to thank Dr. W. H. Longley for his continual help and Mr. H. G. Smith for his assistance.

SUMMARY

1. *Mœandra areolata* is wide-spread at the Tortugas and other Caribbean and Florida reefs occurring on the surface of sand in sheltered areas.

2. Feeding is entirely by means of the tentacles, there is no reversal of ciliary currents.

3. Not only is sediment removed very rapidly from the surface but colonies can completely uncover themselves within twelve hours after being buried under sand.

4. Unlike *Fungia* which uncovers itself by the exclusive action of cilia, *M. areolata* first distends the tissues with water. Distension for cleansing is essentially different from expansion for feeding.

5. Planulation, so far as can be determined at present, has a lunar rhythm, culminating about the time of new moon.

6. After an initial stage when upward and outward growth are about equal, outward growth predominates, an oval or rounded colony with a small basal attachment being finally produced. The stage at which detachment occurs must vary with environmental conditions.

7. Colonies may be formed from a single planula or from the fusion of several. Although the number of individuals which compose a colony can always be determined by an examination of the valleys, the ultimate shape of the colony is not affected.

8. *M. areolata* is a species highly adapted for life on sand occupying in the Atlantic the same habitat occupied by the Fungiidæ in the Indo-Pacific.

9. Adaptibility in reef-building corals is discussed.

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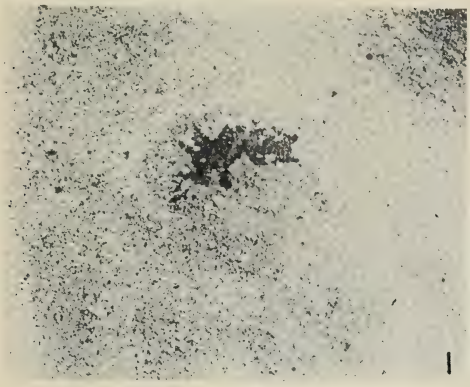


Series of photographs showing gradual emergence of *M. aerolata* after complete burial beneath sand. × 2/5

Fig. 1—Condition 2 hours 20 minutes after being buried.
Fig. 2—Condition 4 hours 25 minutes after being buried.
Fig. 3—Condition 6 hours 20 minutes after being buried.
Fig. 4—Condition 6 hours 55 minutes after being buried.
Fig. 5—Condition 7 hours 55 minutes after being buried.
Fig. 6—Condition 9 hours 10 minutes after being buried.

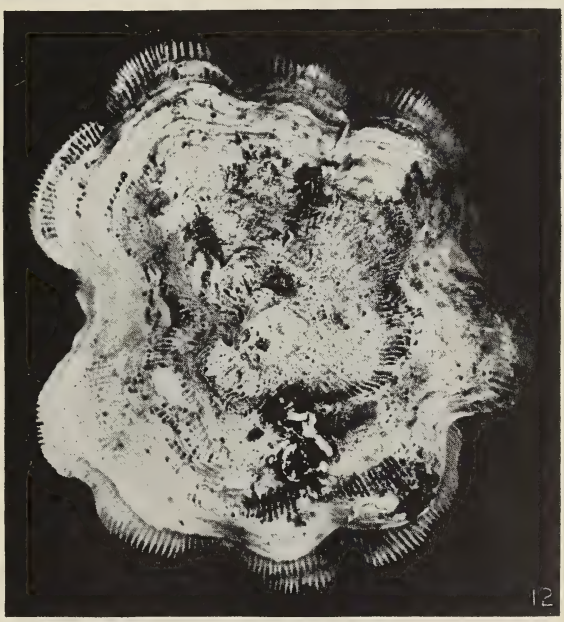
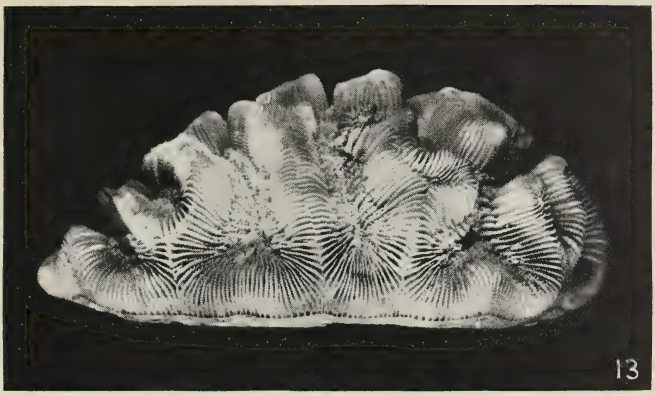


Fig. 7—Young, attached specimen of *M. areolata*. × 1.

Fig. 8—Somewhat older, attached specimen showing outward growth. × 1.

Fig. 9—Specimen with wide basal attachment, due to settlement of planula in a cavity and subsequent fusion with the sides of this. × 1.

Figs. 10, 11, 12—Basal surface of three specimens of *M. areolata* showing progressive reduction of the tissues on the under surface with increased size. × 1.



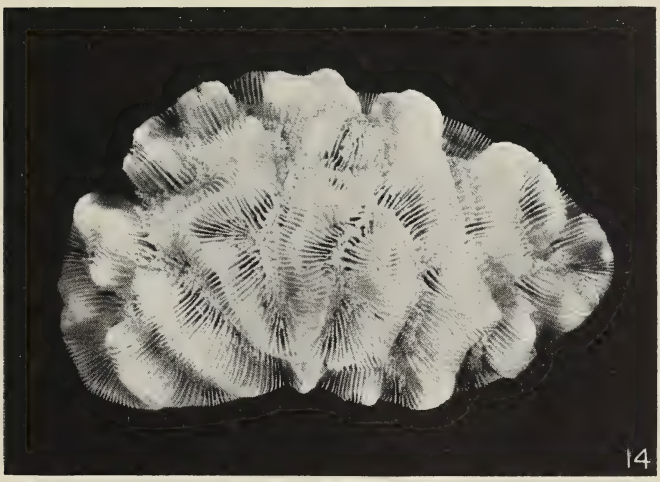


Fig. 13—Side view of a fully grown specimen of *M. areolata* showing complete absence of basal stalk. × ¾.

Fig. 14—Surface view of fully grown colony which has arisen from a single planula. × ¾.

Fig. 15—Surface view of a fully grown colony which has arisen from three planulæ which have fused together. Broken lines indicate the boundaries of the three individuals which compose the colony. × ¾.

PAPERS FROM TORTUGAS LABORATORY, VOL. XXIX

IX

STUDIES ON THE BIOLOGY OF TORTUGAS CORALS

II. Variation in the Genus Siderastrea

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With one plate

[Issued December 9, 1935.]

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STUDIES ON THE BIOLOGY OF TORTUGAS CORALS

II. Variation in the Genus Siderastrea

INTRODUCTION

Vaughan (1902), in his account of the stony corals of Porto Rican waters, describes two species of *Siderastrea, S. radians* (Pallas) and *S. siderea* (Ellis and Solander). He also provides the following tabular summary of the differences between these two species.

S. radians	*S. siderea*
Upper portion of the septal margins, between calices, flat.	Subacute.
Calices deformed: occasionally subhexagonal or rounded.	Subhexagonal.
Corallites, diameters 3 mm. by 2 or 2.5 mm. or even smaller.	5 mm. by 4 or 4.5, occasionally only 3 or 3.5 mm. in diameter.
Calicular fossa, septal margins perpendicular to horizontal plane through bottom of calice.	Septal margins slope to the bottom of the calicular fossa.
Septa, fourth cycle incomplete.	Fourth cycle nearly always complete.
Columella, solid.	Papillary.

He adds "The characters that first strike one's eyes are the relatively smaller calices of *radians*, their more deformed outlines, and the flatness of the upper portions of the septal margins between calices. Besides, *radians* seems to be more explanate in mode of growth and to form smaller masses. *S. siderea* quite often forms rather large heads."

OCCURRENCE ON THE TORTUGAS REEFS

At the Tortugas both of these species are abundant. *S. siderea* is interesting because it probably forms more massive colonies than any other member of the Agariciidæ, a family whose members usually form small colonies, the majority of which are encrusting. Although a few rounded colonies some 10 inches in diameter were seen, partially covered at low-water springs, off the seaward side of Bird Key Reef, the largest colonies, 4 to 5 feet in diameter, were observed while diving in depths of about 2 fathoms off the West of Loggerhead Key.

S. radians, on the other hand, is an encrusting species particularly abundant on the beach-rock on the east of Loggerhead Key. There it forms colonies, oval or round in outline and with an average maximum diameter of

about 6 inches, but only half an inch in thickness at the maximum (Plate 1, fig. 1). It thus offers the minimum of resistance to the water which is continually passing to and fro over it. This coral also frequently settles on loose pieces of dead coral rock, eventually completely covering them and so forming roughly spherical colonies which are rolled about on the surface of the reef. Such colonies were not uncommon on the summit of Bird Key Reef. Verrill (1901) observed similar free colonies at Bermuda, and Duerden (1904) at Jamaica.

As would be expected in view of its habits, *S. radians* is exceptionally hardy. Verrill (1901) states that "Exposure to the dry air, or even to the hot sun, for an hour or so, does not kill it, if it be wet beneath. Probably its porosity enables it to absorb sufficient water to prevent drying up." Mayer (1918) found that out of 8 species of Tortugas corals, which he examined, *S. radians* withstood exposure to the highest temperature, namely 38.2° C., which was 1.15° C. higher than the next most hardy, *Favia fragum*, and 3.5° C. higher than the least hardy, *Acropora muricata*. Wells (1932) has shown that *S. radians* can endure a greater increase in salinity, at least for a limited period (all that is necessary under natural conditions), than the majority of Tortugas reef corals.

There can be no doubt that *S. siderea* and *S. radians* are distinct species. This is indicated not only by Vaughan's list of the differences in the skeleton and by the differences in the mode of growth, but also by the fact the two species may grow side by side. Off the west of Loggerhead Key where *S. siderea* grows to such a large size, typical encrusting colonies of *S. radians* occur attached to the flat rock on the bottom. These colonies do not seem to flourish as well there as in the shallower water where there is greater water movement, possibly because they rely very largely on such movements for cleansing.

OCCURRENCE IN THE MOAT AT FORT JEFFERSON

Interest in this genus was aroused when the fauna in the moat at Fort Jefferson was studied. Vaughan (1918) gives an account of the former coral fauna in the moat. It was then typical of that of the inner reef flats, the commonest species being *Favia fragum, Mœandra areolata, M. clivosa, Siderastrea radians, Porites porites* (= *P. clavaria*), *P. furcata* and *P. astreoides*.

At the time Vaughan made these observations the walls of the moat were intact, a free circulation of water being permitted by openings, one on the west side (marked Flood Gate in Vaughan, 1918, fig. 7), and the other on the east just to the north of the entrance of the fort. The moat was seriously breached on the northwestern side during the hurricane in 1919 and since then has suffered further damage. The result is that today the northeastern, the northwestern and the most northern part of the western sections of the hexagonal moat have been filled, except for occasional pools, with coral

fragments and sand. There is no longer passage for water between the two openings, and in consequence those regions of the moat which are still filled with water (about two-thirds of the whole) constitute a sediment trap. Water enters by way of the eastern opening, all the sediment which it contains falls to the bottom while the tide is in and then the clear water above drains out when the tide falls. On no occasion is the bottom scoured out by water passing through from one opening to the other, and it now consists of very soft mud. This rises when the water is agitated so that the bottom is invisible on stormy days.

This change in the character of the moat has had far-reaching effects upon the fauna, as only animals capable of living on an extremely soft bottom or of resisting the effect of continual and heavy sedimentation can survive. The bottom-living jellyfish, *Cassiopea xamachana*, which used to be abundant has now entirely disappeared. Of the corals listed by Vaughan, *Mœandra areolata* (as recorded in the first paper in this series), *M. clivosa* and *Porites furcata*, have all gone; a very few small colonies of *Favia fragum* and *Porites porites* (*P. clavaria*) were found in a deep pool among the piled coral fragments in the now almost completely blocked northeast moat, but all were in poor condition. In the southeast moat, near to the opening, several colonies of the solid *Porites astreoides* were found, but this was the *only* coral found in the unblocked regions of the moat with the exception of *Siderastrea radians*, which is everywhere abundant on both inner and outer walls.

The survival of this species is not surprising in view of what has been said of its extreme hardiness. Of particular interest is the modification in form which the colonies have undergone as a result, it may not unreasonably be assumed, of exposure to this new environment.

The colonies of *S. radians* can be divided into two groups, according to whether they have grown on the inner or outer walls of the moat. The inner walls, which are those of the fort, are not quite vertical but slope slightly outward, and are largely covered with *S. radians* from low-water level downward. The largest colonies, which are usually oval, have a maximum length of about 1 foot. The calices of these colonies do not differ greatly from those found on the beach rock at Loggerhead Key or on the surface of Bird Key Reef and agree in most respects with the description of Vaughan (1902), but a much more massive skeleton is formed, up to 2 inches in thickness. The corallites are frequently somewhat larger than those of colonies from the Beach Rock; the fourth cycle of septa, though seldom complete, is frequently better developed, while there is a tendency in some cases for the septal margins to slope to the bottom of the calicular fossa instead of being perpendicular.

The outer wall of the moat is vertical, but there is a ledge about 1 foot wide and some 2 feet from the bottom. This is just uncovered at low-water springs. A great deal of alga grows on it, but there are also many

colonies of *S. radians*. The majority of these are on the upper surface but a few, and these the largest, grow over the edge and extend for some distance downward. These colonies differ in a variety of ways from those in other localities; the most striking are those that grow on the upper surface of the ledge. They are frequently hemispherical in shape (Plate I, fig. 2) and have large corallites (fig. 4) up to 4 mm. in diameter. Unlike *S. radians* from the surf region, the calices are never deformed, but strikingly regular and rounded as a comparison of figures 3 and 4 indicates very clearly. The fourth cycle of septa is nearly always complete. Finally the septal margins slope to the bottom of the calicular fossa at an angle of about 45° and there is a very well-developed solid columella. Round the edges of the colony the septa are noticeably thicker and their margins very flattened. They slope at an even less acute angle. In colonies which extend downward, the lower extension resembles the colonies from the inner wall. The wide differences between the shape of the colonies and especially of the calices of *S. radians* in the surf region and on the ledge in the moat are shown in the photographs in Plate I.

It is more than probable that any systematic worker receiving skeletons of these two extreme varieties would classify them as separate species. The extreme variety from the moat differs from the probably equally extreme, but opposite, type from the beach-rock in the following particulars: Rounded and regular calices, larger corallites, sloping septal margins, complete, or almost complete, fourth cycle of septa, and hemispherical skeleton. It differs from *S. siderea* in that the upper portions of the septal margins, between calices, are flat, the calices are never subhexagonal, the corallites never attain the same maximum size, the caliculæ fossa are never more than half the depth (a very striking and obvious difference), the columella is solid, and the colonies are never so massive.

It is clear that Vaughan's summary of the characteristics of *S. radians* needs to be extended to cover the wide degree of variation shown to occur. Moreover an examination of Verrill's plates (1901) of *S. stellata* and especially of *S. stellata* var. *conferta* indicates that these may be still-water varieties of *S. radians*. Verrill himself states that *S. stellata* is "related to *S. radians* and has the same ability to endure impure shallow waters and exposure to air and sunshine, without injury." Rathburn (1879) states that *S. stellata* occurs in "the tide pools of the rocky surfaces, and just below the level of low tide" in the Bay of Bahia on the coast of Brazil. His plate of the reef shows that the pools on the landward side are protected from the surf and that in these *S. radians* would tend to assume the still-water form characteristic of the moat at Fort Jefferson.

The great variability in form of *S. radians* was demonstrated by the large number of colonies obtained from the ledge in the moat which showed gradations from the one type to the other. In these cases the uppermost portion of the skeleton resembled that of the variety from the beach rock,

while around the margins the calices were of the other type. There can be no question that we are dealing with the same species which is modified by the habitat. Some indication of this is also provided by the colonies on the inner wall, which, as always stated, are less explanate in form than those from outside and tend to have somewhat larger corallites with a greater number of septa.

SIGNIFICANT DIFFERENCES IN THE ENVIRONMENT

There are three possible factors which may be responsible for this change in form. These are temperature, water movement and sediment, the two latter being probably associated. Of these, temperature can probably be ruled out. Dr. F. C. Steward kindly supplied the information that on July 22, 1934, a very still hot day, the temperature of the water in the moat (in the sun) was 33.5° C. whereas outside it was only 0.5° C. lower.

For reasons which have already been discussed, water movements are much less and the fall of sediment much greater within the moat than outside. These are the very factors which would be expected to have the greatest influence on the form of sessile animals growing vegetatively. The presence of colonies of *S. radians* both on the inner wall of the moat and also on the ledge on the outer wall makes it possible to attempt to separate the effect of these two factors. The colonies growing in the former locality are not exposed to violent water movements like those from the beach rock but, owing to the almost vertical surface to which they are attached, they will probably not be greatly influenced by the large amount of sediment, because gravity will assist in its removal. It is not, therefore, surprising to find that they differ from colonies from the beach rock primarily in their less explanate form. It may not unreasonably be assumed that the excessively explanate form of the latter is the result of the rapid and continuous backward and forward water movements to which they are exposed.

The colonies on the ledge, on the other hand, are exposed to a continual rain of sediment. It is possible that this provides the explanation for the still more rounded form of the skeleton and also for the increased size of the calicular fossa. It is improbable that the latter are the direct result of the rounded surface and the greater opportunity thereby provided for growth of the individual calices, as some rounded colonies were found with small calices typical of the flattened colonies from the beach rock.

An attempt was made to discover whether the rounded colonies from the moat were better adapted for the removal of sediment than the flattened colonies from the beach rock. In the genus *Siderastrea* the cilia on the disc carry particles away from the mouth, these collect in strings between the polyps and are eventually removed. At night, when the polyps expand, the tissues are raised about 1 to 2 mm. above the surface, and those of adjacent polyps overarch the intervening region so that the outgoing tracts upon them are obscured. Duerden (1902, 1904) and Vaughan (see notes

in Yonge, 1930) have described the shape of the expanded polyp and both have noted the remarkable efficiency of the tentacles which can even capture small jellyfish and annelids. These statements were abundantly confirmed.

Rounded colonies from the moat and flattened colonies from the beach rock were placed in large jars containing 2½ litres of sea-water. To this had previously been added 2 grams of dried mud obtained from the moat and strained through a sieve of mesh 100 to the inch. This fine sediment settled very slowly and it was not possible to observe the colonies clearly until the end of 18 hours. At the end of that period the specimens from the beach rock had a great deal of mud, mixed with mucus, on their surfaces and were clearly experiencing considerable difficulty in ridding themselves of it. The specimens from the moat had cleared themselves much more satisfactorily, only a few areas being covered with sediment. After 24 hours a great deal of sediment was still present on the surface of the former colonies, but the latter were almost completely clean. Very similar results were obtained when the surface of the colonies was covered lightly with fine sand. There was also a very noticeable difference between the speed of removal of carmine particles by the two types of colony when this was observed under the binocular microscope.

Finally four colonies of *S. radians* from the beach rock were removed and placed on the ledge in the moat. This was unfortunately carried out late during the period spent at the Tortugas and it was only possible to observe them for 12 days. At the end of this period, however, they were all covered with strings and accumulations of fine muddy sediment, whereas the colonies which had grown on the ledge seldom had any trace of sediment upon them. There seems no doubt that the rounded colonies in the ledge in the moat are much better adapted for life in water which contains a great deal of sediment than are the flattened colonies from the beach rock.

DISCUSSION

The subject-matter of this paper raises anew the vexed question of the relation between form and environment in corals. More than a generation ago, Hickson (1898) came to the conclusion that in the Hydrocorallinæ, the genus *Millepora,* despite the innumerable forms which it takes, consists actually of the single species, *M. alcicornis,* which is Indo-Pacific as well as Atlantic in its distribution. Wood-Jones (1907, 1912) emphasized the fact that the form of Madreporaria is frequently conditioned by environmental factors and believed that the number of species had been greatly exaggerated. Vaughan (1919) gives certain support to this view. The matter has recently been extensively discussed by Stephenson (1933) as a result of his work during the Great Barrier Reef Expedition. His discussion is too long to be summarized here, but should be read by all interested in this matter. He comes to the general conclusion, as a result of observational and

experimental work, that "species in the ordinarily accepted sense of the term do exist in many coral genera in considerable number, and that many of them may be fairly easily recognized in the field; and that Wood-Jones has considerably overstressed the effect of environmental conditions on the corals, although such an effect certainly exists, and is responsible for a considerable range of variations."

The observations here recorded on the genus *Siderastrea* point to a similar conclusion. There can be no doubt as to the specific distinction between *S. radians* and *S. siderea,* but the former is capable of wide variations which fit it for life in a variety of habitats. The change in conditions in the moat at Fort Jefferson has afforded a striking demonstration of this. Of the few species which have managed to survive exposure to the new conditions, only *S. radians* has flourished. This it has done because it is essentially a shore-living species with the physiological adaptations characteristic of all such, namely the capacity to survive high temperatures, variable salinities and desiccation, but also because it has a remarkable power of modifying the skeleton according to the environment. There modifications affect the shape of the skeleton, the size and shape of the corallites, and the number, slope and thickness of the septa. Thus it is that the same species can flourish in the surf on the beach rock and in the still, sediment-laden water in the moat. The specific identity of these two extreme forms is proved by the presence of every possible gradation between the two, not only in different colonies but, so far as the shape of the calices and the nature of the septa are concerned, in one and the same colony. In view of these facts the specific identity of *S. stellata* Verrill would appear to require critical examination.

In the preceding paper in this series an account was given of the high degree of specialization possessed by *Mœandra areolata* which enables this animal, like the Fungids of the Indo-Pacific, to live on a sandy bottom. In contradistinction to this rigid specialization there is the wide range of modification of which *S. radians* is capable and which permits it to flourish under a variety of conditions. The great success of the Madreporaria, which is so forcibly demonstrated by the wide-spread occurrence and immense size of coral reefs, may not unreasonably be attributed to the presence within the group of species highly specialized for a particular environment, and others capable of wide modification in form which enables them to adapt themselves for life in a variety of different environments. The acceptance of this view would certainly explain, and perhaps tend to allay, the conflict between those who believe in the validity of the great number of species of Madreporaria which have been described and those who regard the majority of these as no more than growth forms.

Acknowledgments are due to the Carnegie Institution of Washington, to the Royal Society of London and to Dr. W. H. Longley, for reasons recorded in the first paper in this series.

SUMMARY

1. The genus *Siderastrea* is represented at the Tortugas by the two species, *S. siderea* and *S. radians*.

2. *S. siderea* forms larger rounded colonies which were not observed above the level of low-water springs.

3. *S. radians* is essentially a shore-living species possessing the physiological adaptations characteristic of all shore-living animals.

4. *S. radians* is capable of great modification both in the form of the skeleton as a whole and also in the size and shape of the corallites and in the number, slope and thickness of the septa.

5. This species has been enabled, as a result, to occupy a variety of habitats, the extremes being represented by the surf region on the beach rock on the one hand, and by the still, sediment-laden water in the moat at Fort Jefferson on the other.

6. The relation between form and environment in corals is discussed and the general conclusion reached that the great success of the Madreporaria is probably due to the presence of species highly specialized for a particular environment and also of others which can be modified for existence in a variety of different environments.

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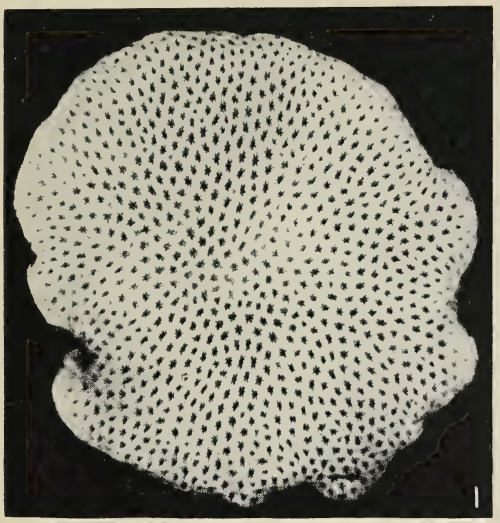

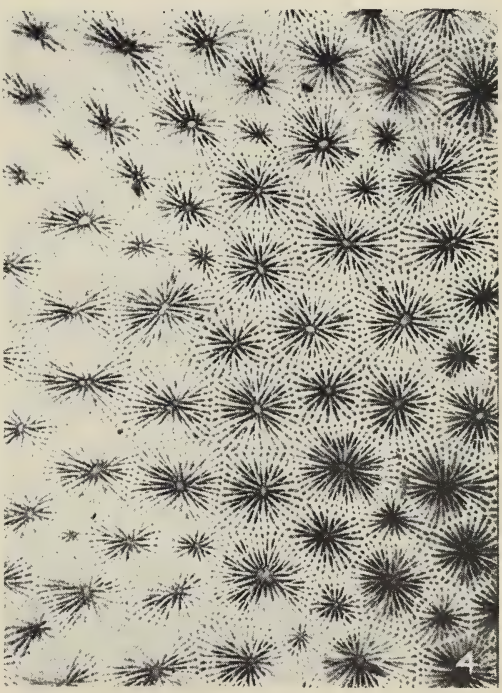

Fig. 1—Upper view of a typical colony of *S. radians* from the surf region on the beach-rock to the east of Loggerhead Key. This colony has a maximum diameter of 9 cm. and a maximum thickness of 1 cm.

Fig. 2—Side view of a colony of *S. radians* from the ledge along the outer wall of the moat at Fort Jefferson. The colony is hemispherical with a maximum diameter of 11 cm. and a maximum thickness of 4 cm.

Fig. 3—Enlarged photograph of the surface of the colony shown in figure 1. x 4.

Fig. 4—Enlarged photograph of the surface of the colony shown in figure 2. x 4.

PAPERS FROM TORTUGAS LABORATORY, VOL. XXIX

X

METAMORPHOSIS OF ASCIDIAN LARVÆ

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With two plates and one text-figure

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METAMORPHOSIS OF ASCIDIAN LARVÆ

GENERAL OUTLINE

The studies of metamorphosis of larvæ of ascidians, an account of which is given in this paper, were made during the Summers of 1927 to 1930 and 1933 at the Tortugas Laboratory of the Carnegie Institution of Washington, with the purpose of finding methods of accelerating and controlling metamorphosis and thus of discovering something of the fundamental nature of the internal mechanism involved and the environmental conditions with which it is causally related.

They were preceded by many years' work with ascidian larvæ of several species at the Marine Biological Laboratory, Woods Hole, in part concerned with the duration of their free-swimming periods. This early work, like that of 1926 at the Tortugas Laboratory, was done on the generally accepted assumption that the duration of this period is as specific and as definitely fixed in the ontogeny of each species of ascidian as that of any morphological stages or physiological processes running their course in a relatively constant conditioning environment. The results of these earlier investigations, published in a series of papers (Grave, 1920, 1926; Grave and McCosh, 1923; Grave & Woodbridge, 1924), seemed consistent with the assumption that the extreme variability found in this character is due to genetic rather than to environmental differences.

Observations made while studying the larva of *Symplegma viride* in 1927 seemed to show that an environmental factor profoundly influences the time of occurrence of metamorphosis. Since these became the starting point of the experimental studies now reported, they are described here in detail.

Twenty-two larvæ, liberated by the parent colony on the morning of July 15, and 51 larvæ liberated on the 16th, gave expected results, 27 and 40 per cent, respectively, having metamorphosed after free-swimming periods varying from 2.5 to 10 hours. But on the 17th, none of 48 larvæ taken from a brood liberated by the same parent colony had metamorphosed before the 10th hour of larval life, and all died the following day without metamorphosis. This was the second intimation that broods of larvæ, liberated by the same *Symplegma* colony on different days, may vary in the percentage of larvæ that metamorphose in a given time to an extent much greater than can be accounted for on any theory of chance distribution of a genetically determined character within a normal curve of frequency. An earlier intimation that environmental conditions may be involved in determining the occurrence of metamorphosis came on the morning of the 16th. On this occasion when 90 *Symplegma* larvæ were being carried in a 50-cc. beaker from one well-lighted part of the laboratory to another (less lighted), 50 of the larvæ first showed unusual behavior and activity,

then attached themselves to the side and bottom of the beaker and metamorphosed one by one in rapid succession after a free-swimming period of not more than 1.5 hours.

What could be the cause of this unexpected behavior? Of conditions and events during the period involved note was made of all that could be recalled: July 15 and 16 were days of bright sunshine, clear atmosphere and high temperature; July 17 was relatively cool and dark, the sky being overcast throughout the day. The coincidence of these differences in light conditions with wide differences in the time and percentages of metamorphosis, considered in connection with the epidemic of metamorphosis that occurred in the beaker on the 16th, suggested that *light* is perhaps the environmental factor responsible in some way for releasing the process of metamorphosis. With this possibility in mind, experiments were made in which groups of larvæ were variously exposed to sun-light, to shadow and to darkness in an attempt to reproduce the conditions that had caused the epidemic of metamorphosis in the beaker on the 16th. Some of these experiments were sufficiently successful (see page 228) to suggest the hypothesis that a direct quantitative relation exists between duration of the free-swimming period of a larva and the amount of light to which it is exposed. In a report of this work (Grave, 1927), the further assumption was made that ascidian larvæ may contain a supply of a photochemical substance, variable in quantity, which inhibits metamorphosis, and that light decomposes or destroys this substance thereby releasing the process of metamorphosis.

The results of experiments made in 1928 gave little support to these assumptions, but in the various tentative hypotheses formulated to account for metamorphosis *light* continued to be regarded as a necessary and specific agent until it was suggested by the concluding experiments of 1929, and proved by those of 1930, that metamorphosis is induced in "susceptible" larvæ by any influence that stimulates them to continuous activity, and apparently in direct proportion to the extent of the swimming activity thereby induced. Metamorphosis was shown by these experiments to be caused, not by the direct or specific effect of any method of treatment on an internal mechanism of metamorphosis, but by a reaction involving some by-product of the neuromuscular activity to which larvæ are stimulated by experimental treatment.

Despite the fact that light is not the specific agent by which metamorphosis is induced and does not act directly on the internal mechanism of metamorphosis, still it is the most effective physical agent by which larvæ may be artificially stimulated to activity and it is the form of energy to which larvæ are almost exclusively responsive in nature. By the possession of a sensory mechanism extremely sensitive to stimulation by light, the types of larvæ with which this investigation has dealt are by structure adapted to and dependent upon light for the external stimuli required to initiate and

orient their swimming activities. These larvæ also possess a statolith sensitive to changes in the position of the larva which, in the moving water of the open sea, may often stimulate them to swim, but this mechanism is much less sensitive than the light-perceiving organ and probably functions more in orienting swimming movements than in inducing them.

A bias in favor of light as the specific agent required to initiate the process of metamorphosis was created or has been suggested by the initial observations of this investigation. Strengthened by the remarkable and continuous success attending experiments in which changes in light intensity constituted the method of treatment, it was too strong to permit easy acceptance of the repeatedly suggested hypothesis that all this induced metamorphosis had been caused, not by light to which the larvæ had been exposed, but by the activity it evoked. Furthermore, many observations and experimental results were in direct conflict with the over-simplified hypothesis that a direct relationship exists between the amount of larval activity and the percentage of metamorphosis that takes place. It was, therefore, not until the work of 1930 had ended and a more thorough study of available data had been made, that these conflicts were resolved in favor of the hypothesis now held, which ascribes to metabolic products of larval activity a real but not an exclusive or primary function in causing metamorphosis.

This final hypothesis, which is in harmony with all known facts, assumes the necessity of another substance with which the metabolic by-product of larval activity must combine before it can be effective in the induction of metamorphosis. Without this other substance to which the name *susceptibility* substance or factor has been given, larval activity is without effect. No amount of activity is able, of itself, to induce metamorphosis.

The existence of such a susceptibility factor first became evident during 1929, when the same *Polyandrocarpa* colony produced brood after brood of larvæ that showed extreme variation in the amount of experimental treatment required to induce metamorphosis. The larvæ of one of these broods metamorphosed to the extent of 67 per cent after treatment consisting of 20 one-minute exposures to light, each followed by a one-minute exposure to darkness (Experiment 77, page 236), while those of other broods required the same kind of stimulation extending over many hours (Experiments 81 and 121, pages 244 and 248). In the larvæ used in Experiment 77 the susceptibility factor was present almost immediately after liberation; in those of Experiment 121 it had not appeared after seven hours.

Evidence is provided by certain other observations, that the susceptibility factor does not persist indefinitely after having been formed, but waxes and wanes during the free-swimming period, finally disappearing or disintegrating, and that when it disappears the larva loses its ability to metamorphose. Presumably about 90 per cent of larvæ of vial 2 in Experiment 53 (page 233) would have metamorphosed had they during the hour beginning at

about 12:30 a.m. been stimulated to activity equal to that of the larvæ in vial 3, but six hours later, at 5:30 p.m., 44 per cent only could be induced to metamorphose. It was frequently observed that larvæ kept inactive for relatively long periods usually died without metamorphosis.

To a considerable extent the formation of the metabolic byproduct and the amount formed are subject to control through control of the activity of larvæ, but nothing is now known of the origin of the hypothetical susceptibility factor. In larvæ of the *Botryllus* type, no evidence has been found that production of the latter is either accelerated or inhibited by environmental factors, but certain experiments with larvæ of *Phallusia* indicate that its formation is influenced by external events—metamorphosis in this larva occurs to the extent of 100 per cent in less than two hours if the larvæ are greatly crowded, but when not so crowded metamorphosis may be delayed five days or longer.

Experiments made during 1933, in which larvæ of both types were treated with various chemical and biological substances, gave results which seem to indicate that substitutes for each hypothetical factor constituting the mechanism of metamorphosis may be approximated. Many of these substances greatly accelerated metamorphosis in ways suggesting that some had an effect like that of the susceptibility factor, others an effect like that of activity and some seemed to act directly upon the larval nervous system.

Below, in order of succession, are the working hypotheses, which during this study have been formulated tentatively concerning the causes of metamorphosis and their mode of action.

(1) 1927-1928. Metamorphosis is induced by the destructive or transforming action of sunlight on some photo-chemical substance which inhibits metamorphosis.

(2) 1928-1929. Sudden changes between two levels of light intensity, made at intervals of about 1 minute, induce metamorphosis by causing reversible reactions in some photo-chemical substance; energy being liberated when the change is from a higher to a lower level of light intensity and being absorbed when the change is from a lower level to a higher.

(3) 1929. Metamorphosis is induced by "shock" reactions resulting from any series of sudden stimulations of the larva by light, heat, electricity or mechanical disturbance. These shocks were assumed to act in a way to disturb some balanced equilibrium between the chemical links in the chain that constitutes the mechanism of metamorphosis.

(4) 1929-1930. Metamorphosis is induced by larval activity which tends to use up some larval energy-reserve the presence of which prevents larval disintegration; but the effect of larval activity is in some way modified or inhibited until some substance (the susceptibility factor) is produced by the larva subsequent to liberation by the parent.

(This was not regarded as a complete hypothesis. It was known that at least two factors were affecting the process of metamorphosis, but in what way was not visualized).

(5) 1930-1933. Metamorphosis occurs when an internal secretion, produced by some larval organ or tissue, reacts with some by-product of metab-

olism of the larval neuro-musculature (produced during swimming activity) to form a third substance that acts directly and progressively on the larval nervous system, causing (1) adaptive changes in responses of the larva to light and "gravity"; (2) attachment of the larva; (3) spasmic, tetanus-like stimulation of the muscle bands to vigorous contractions by which the entire action system of the larva is crumpled, disrupted and withdrawn into the posterior region of what is now the body of the ascidiozooid.

Much of the work done prior to the summer of 1930 as is evidenced by this list was planned in accordance with the implications of hypotheses now known to be invalid, but some results then obtained still have interest. In the slow approach to a more correct view of the nature of metamorphosis they marked progress. Hence all findings upon which the final analysis is based are given in the pages following this introductory statement of the problem and general account of the approach to its solution. The order in which some aspects of the subject are arranged is not logical but follows that in which they developed during the investigation.

For the opportunity to make this extended study under the ideal conditions provided by the Tortugas Laboratory and the rich ascidian fauna in its vicinity, I am indebted to the Carnegie Institution of Washington and especially to the Executive Officer, Dr. William H. Longley, who has followed the investigation with an active interest and has provided every facility required to carry it to a satisfactory conclusion. From Captain John Mills most valuable aid has been received in the construction of equipment. Acknowledgment is also made to Dr. S. O. Mast and Dr. M. Luckish for technical information and advice concerning equipment and methods for work with light. Dr. W. G. VanName, of the American Museum of Natural History, has kindly identified species of ascidians with which the work has been concerned and has generously associated my name with the species of *Polyandrocarpa* new to science. During the summer of 1933 I was assisted by Mr. Paul A. Nicoll, to whom I acknowledge indebtedness for active cooperation in the planning and execution of a long and difficult program of experiments which involved the principles and methods of physical chemistry and general physiology.

MATERIAL AND METHODS

An abundant and constant supply of larvæ of the same age and parentage, having a relatively long free-swimming period and produced under physical and biological conditions that remain practically uniform throughout the period of investigation, is a requirement for an experimental study of metamorphosis almost ideally met by several species of compound ascidian of the Tortugas region (*Symplegma viride*, *Polyandrocarpa tincta* and *P. gravei*) and by one species of simple ascidian (*Phallusia nigra*). These species of compound ascidian are further suited to the needs of such a study in that mature colonies taken from their natural habitat thrive in

a live-car anchored near shore and may be transferred daily to aquaria in the laboratory for several hours without injury.

Species whose larvæ have free-swimming periods of short duration are legion in almost all regions where ascidians are found but they are obviously unsuited to experimental studies involving changes in the environmental conditions by which metamorphosis is accelerated or inhibited, since it is not possible with larvæ of these species to distinguish metamorphosis occurring normally during the period of experimentation from that induced by experimental means.

Brief statements concerning certain characteristics of the adult and larval forms of the species that provided material for this study are made in this connection, partly as an aid to any who may desire to make use of the same species for work in the future, but chiefly because the close correlation that exists between the activities and responses of an ascidian larva and the place and mode of life of the adult form are facts that must be considered in any attempt at analysis of such adaptive behavior and processes as accompany and constitute metamorphosis.

Wild colonies of *Symplegma viride, Polyandrocarpa tincta* and *P. gravei* almost without exception are found encrusting the underside of dead coral rock, empty shells of mollusks and especially pieces of sheet-iron and slate that lie scattered on the sandy and rocky bottoms west and northwest of Fort Jefferson over which the water at low tide has a depth varying from about one to five feet. These species incubate their eggs in the atrial cavity and give birth periodically to fully developed free-swimming larvæ, drawings of which are reproduced in Plate I. The dimensions of these larvæ, which belong to the *Botryllus* type in structure and behavior (Grave 1932), are as follows:

	Total length	Body length	Tail length
Symplegma viride	1.55 mm.	0.40 mm.	1.15 mm.
Polyandrocarpa tincta	1.13	.27	.86
Polyandrocarpa gravei	1.35	.30	1.05

When larvæ of these species of compound ascidian were desired, a colony from the live-car was transferred to a large glass bowl of fresh sea-water in the laboratory usually between 7 and 8 o'clock in the morning and placed on a table before a window facing west. At frequent intervals the surface of the water nearest the window was scanned for swimming larvæ, for as these were liberated they accumulated at this most illuminated part of the bowl and were easily collected by means of a wide-mouthed pipette and transferred to beakers or shell vials.

A large colony of *Symplegma viride* could usually be relied upon to liberate larvæ almost as soon as it had been placed in the bowl and to continue to set them free in a more or less constant stream throughout the day, but unfortunately this species is quite rare at the Tortugas, and during the seasons of 1929, 1930 and 1933 none was found. Both species of *Polyandrocarpa* are abundant and several large growing colonies supplied larvæ for

work during each of these seasons. They tend to liberate larvæ in a swarm once only during the day, usually between 11 a.m. and 12:30 p.m., but in one instance a swarm was released as early as 8:30 a.m. and in another as late as 3 in the afternoon. A large mature colony will sometimes liberate swarms of larvæ on two consecutive days but none was known to produce a swarm three days in succession. Reference is made to this fact because of its bearing upon the problem of the age of larvæ of a brood, whether it consists of larvæ all of the same age or whether it may not contain larvæ of different ages derived from eggs fertilized at various intervals following the liberation of the previous brood. The liberation of a brood or swarm of *Polyandrocarpa* larvæ is a continuous process covering a period varying from about 20 to 40 minutes. The number of larvæ constituting a swarm varies greatly with the size and maturity of the parent colony, from less than 50 to more than 1000, a swarm of average size numbering about 300.

The large black *Phallusia nigra* is found sparingly on many of the bottoms in the Tortugas region attached to coral rock and stems of gorgonians but is abundant on the brick walls within the moat at Fort Jefferson. Having been torn from their attachment, individuals soon die but may be kept in a live-car for a day or two without deterioration of their eggs and sperm. Fertilization of the eggs and development of the relatively small larva shown in Plate 2 take place outside the body of the parent. This larva has an average total length of about 0.93 mm., body length 0.18 mm., tail length 0.75 mm. Development is exceedingly rapid. At temperatures between 27° and 29° C., eggs fertilized at 5 a.m. yield free-swimming larvæ at about 1 p.m., and one lot hatched after a period of development of only 6 hours and 38 minutes (Grave, 1925). By fertilizing eggs taken from several individuals early in the morning it is possible to have many thousands of free-swimming larvæ available for experimentation during the afternoon.

The procedure followed with success is simple. After cutting away the projecting ends of the siphons incisions were made in the tunic between the siphons and along one side from siphon to base of body. Spreading the opening thus made and seizing the stump of one siphon with forceps, the body was pulled away with no injury save to blood-vessels. Removed from the tunic, washed with fresh sea-water and placed separately in dry petri dishes, animals were left until the blood from severed mantle vessels had clotted. Since the long genital ducts lie close together near the surface at one side of the body, and when filled with eggs or sperm are plainly visible, it was then possible to puncture the oviduct near its opening into the atrial cavity, without rupturing the sperm duct, provided the puncture was made with a very sharp needle. As eggs flowed from the puncture they were taken up with a wide-mouthed pipette and transferred to beakers of fresh sea-water and later fertilized with sperm taken either from the same or a different individual. Especial care had always to be taken that no blood was included

with the eggs or sperm, as even slight contamination results in abnormal development.

For experimental studies in which the statistical method of measuring results must be applied, it is desirable that the factors of individual variation be reduced to a minimum. This desideratum is approached in larvæ of ascidians when each experimental lot is composed of individuals having the same percentage; even self-fertilization adds to the desired uniformity. Eggs of different parentage develop at rates sufficiently different to permit one lot to hatch several minutes before or after another, and undoubtedly they vary more or less in all hereditary characters.

It was often necessary to divide eggs taken from a single individual and to place them in two or more beakers, for when too many eggs are crowded together during development it was found that abnormalities appear during the later stages often to the extent of 100 per cent depending upon the extent to which they are crowded. Without attempting to ascertain just what constitutes crowding and what quantity of eggs in a 50 cc. beaker is the optimum for normal development, it was found empirically that abnormalities are likely to occur when the bottom of the beaker is covered by a layer more than one egg in thickness. To free the eggs from excess sperm and prevent the accumulation of injurious by-products of development, the surface stratum of water in each beaker was decanted and replaced with fresh sea-water as soon and as frequently during the first three or four hours after fertilization as settling of the eggs would permit. After this, less frequent changes in the water were similarly made.

The problem of high temperatures at the Tortugas Laboratory has been a serious one for all whose work requires the keeping of eggs and small marine organisms in a normal condition for relatively long periods. In small vessels the temperature of sea-water in the laboratory, especially on the warmest days, often rises as high as 34° C., at which level many organisms fail to react normally and are permanently injured.

This problem was satisfactorily solved in 1927 when Captain John Mills devised what is known at the Laboratory as the Mills Water Table, a brief description of which was published in the Carnegie Institution Year Book No. 26 (Grave, 1927). It has been used throughout this investigation. It consists of a shallow zinc pan made to fit the top of a laboratory table upon which it rests; about 3 feet in width, 8 feet in length and 4 inches in depth, with outlets at one end, one near the bottom and one at the 3-inch level. At the opposite end a 5-sided, tent-shaped frame work, about 5 feet in height and 3 feet in diameter at the base, was erected and covered with cheese cloth. At the apex of the tent a sprinkler continuously sprayed the cheese cloth with sea-water and provided a supply of water for the pan. By evaporation from the surface of the tent and of the water that filled the pan to a depth of 3 inches, a temperature in the pan was maintained throughout the warmest days at about that of the open water at the laboratory wharf

and never above 30° C., even when the air temperature of the room reached 38° C. At night, if the spray was not turned off, the temperature of the water in the pan fell considerably below that of water at the wharf.

Vessels placed in the water of this table maintained a temperature the same as that of the water surrounding them. Concrete blocks of the proper thickness, placed in the pan, supported panes of plate glass at a level a few millimeters below the surface of the water. These when covered with sheets of black paper served as tables for supporting small beakers and shell vials used as containers of developing eggs and of larvæ during experimentation.

A method of temperature control used during treatment of larvæ with a beam of direct sun-light from a heliostat, and with light from electric light bulbs in a light-proof exposure box, consisted in fitting several coils of glass tubing to the inside wall of a small rectangular glass vessel through which a stream of cooled water flowed during the period of an experiment. The temperature of the water of the vessel and therefore of the vials containing larvæ standing in this cooled water was thus kept from rising to lethal levels.

EXPERIMENTAL TECHNIQUE

In the experiments first made, larvæ were exposed to treatment in 50 cc. pyrex beakers but in all work done since 1927 shell vials of various sizes have been used. The type of vial best adapted to work when accurate counts of free-swimming and metamorphosed larvæ were desired at frequent intervals during and after treatment is one having a height of 6 cm., a diameter of 1.8 cm. and a capacity of about 10 cu. cm. when filled to a level about 0.5 cm. below the top. Larvæ on the bottom of a vial of this size are plainly seen and counted with a watchmaker's lens.

When counts of larvæ and ascidiozooids may be deferred until the close of an experiment, the type of vial preferred has a height of 8 cm., a diameter of 2.5 cm. and a capacity of 27 cu. cm. when filled to a level about 1 cm. below the top. This is the size of vial used in all experiments made with *Polyandrocarpa* larvæ during the summer of 1933 when experimental treatment involved the addition of various substances and extracts to the sea-water medium, but the smaller vial was used in all experiments of this kind with the larva of *Phallusia*.

In preparing for an experiment, all vials to be used were thoroughly cleaned first with fresh water, soap and brush, then rinsed in fresh water, and at last with ethyl alcohol. An hour or so before they were to be stocked with larvæ the vials were rinsed repeatedly with sea-water and then filled with sea-water until larvæ were available.

The number of vials used for an experiment was never less than three, one containing the larvæ to be experimentally treated, two for controls. As the work progressed and the extreme variability of larvæ became more evi-

dent, the number of larvæ and the number of vials for each experiment were increased to at least 3 for each type of treatment and 3 for the control. Experiments also increased in diversification of treatment until, in 1930 and 1933, each experiment included as many as 15 to 24 well-stocked vials, 3 for each of several methods of simultaneous treatment.

The method of stocking vials for an experiment with larvæ of *Symplegma* or *Polyandrocarpa* is as follows: all vials to be used were partially filled with sea-water and placed in a planned arrangement on the Mills Water Table until a brood of larvæ was in process of liberation. When larvæ began to appear at the most illuminated edge of the bowl in which the colony of adult ascidians had been set, they were taken up with a pipette, as they accumulated, and transferred to vials, until each was estimated to contain 50 or more larvæ. This was continued until all vials were thus stocked or until larvæ ceased to be liberated, record being made of the exact time when each vial was stocked. Water was added so that all vials contained the same volume. This operation extended over the 15 to 30 minutes required for the liberation of a brood.

The vials having been stocked, experimental treatment began and was continued during a planned interval or until cases of metamorphosis began to occur at frequent intervals in the vial or group of vials subjected to the method of treatment considered most effective in inducing metamorphosis. Treatment was then discontinued and formalin was either immediately added to all vials, thus ending the experiment, or the vials stood in place for a time to permit the treatment to have its full effect. Counts of the number of larvæ were then made and the percentages of metamorphosis calculated, the contents of each vial having been emptied into a watch crystal to facilitate the counting operation. The several methods of treatment used during the investigation are described in connection with sections dealing with experiments and results.

The method of handling and distributing the small *Phallusia* larvæ is somewhat different from that just stated. Shortly before a lot of *Phallusia* eggs was expected to hatch, all vials to be used in an experiment were arranged in a planned order in the Mills Water Table and a quantity of sea-water was placed in each, just sufficient to amount to 9 cc. when a measured pipette full of larvæ was later added. When the most illuminated part of the water surface of the beakers, in which a lot of eggs had developed, was swarming with thousands of swimming larvæ, they were carefully skimmed from the surface with a pipette and transferred to another beaker, pipette full after pipette full, until a sufficient volume to stock the vials had thus been concentrated. Then keeping the larvæ agitated and thus equally distributed in the beaker, they were transferred one pipette full to each vial. They were now ready for treatment.

In all experiments made prior to 1933 nothing was added to the sea-water medium. The treatment always followed upon segregation of larvæ

into vials and was entirely physical in nature, but in 1933 the treatments were mainly chemical and the substances and extracts used were usually added to the water of the vials before larvæ were introduced.

ACTIVITY AND BEHAVIOR OF ASCIDIAN LARVÆ

Larvæ of ascidians of the types used in this investigation exhibit a behavior during the free-swimming period that is stereotyped but variable. Visible changes occur in orientation to environmental stimuli that are doubtless conditioned by corresponding structural and physiological changes, some or all of which seem to have a close correlation with internal events related to the process of metamorphosis.

A larva of *Symplegma viride*, *Polyandrocarpa tincta* or *P. gravei*, at the time of liberation from the brooding parent, or of *Phallusia nigra*, at the time of hatching from the egg membrane, is stimulated by its first contact with pure sea-water to make its first relatively long burst of swimming activity. Its swimming movements at this time are oriented upward by a negative response to "gravity," and toward the most illuminated part of the vessel by its positive response to light. Directed by the resultant of these responses it swims continuously at the surface of the water nearest the source of light for a relatively long interval; about 10 to 15 minutes, or apparently until exhausted. Then becoming motionless it sinks slowly to or toward the bottom. Intervals of similarly oriented swimming movements now alternate with intervals of rest, the periods of spontaneous activity gradually becoming shorter, the periods of rest gradually increasing in duration. During the early part of the free-swimming period, the periods of rest are momentary and do not afford time for the motionless larva to sink to the bottom, the duration of the periods of spontaneous activity greatly exceeding that of the periods of rest, but the periods of inactivity gradually become longer and soon exceed those of activity. During a longer or shorter series of these recurrent periods of activity, the larva continues to show the original negative response to gravity and the same positive response to light. Each time it swims it rises immediately to the most illuminated part of the surface, swims at this level for a few moments, becomes inactive and sinks to the part of the bottom most illuminated. Under the monotonous conditions of light and stagnation of water in the laboratory, larvæ may never advance beyond this phase of their responses. Their spontaneous activity ceases and they either die without metamorphosis or it occurs relatively late and is not accompanied by its adaptive aspects. If, however, larvæ are stimulated to activity at frequent intervals by interrupting the light with some opaque object, or better, if they are treated by the method that proved to be most effective in their activation, described on page 230, changes in the orientation of swimming movements finally appear. Sooner or later the larvæ, in gradually increasing number, swim upward to the *least illuminated* part of the surface of the vial at each

stimulation, then downward to the *least illuminated* part of the bottom which they actively explore before coming to rest. These visible changes in orientation of movements came to be recognized as infallible signs that the larvæ exhibiting them were near the end of the free-swimming period and could be expected with the next few intervals of activity to swim downward vigorously for a last time, attach themselves to the least illuminated side or bottom of the vial and pass through the disruptive phase of metamorphosis. This relationship between the time of reversal of orientation to light and gravity and the occurrence of metamorphosis is accepted as showing either a common mechanism for both or a very close structural and functional correlation.

Other aspects of larval behavior, exhibited during experimental treatment in which light intensity changes are made at intervals of one minute, are interpreted as showing that the internal effect produced by a sudden change from a higher to a lower intensity is different from the internal effect of a sudden change from a lower intensity to a higher. Invariably, when the larvæ in a vial are suddenly flooded with light of relatively high intensity there is no movement for an interval varying from about 3 to 10 seconds. During this brief latent period all larvæ remain motionless on the bottom where they had settled before the close of the preceding period of activity; but after the latent period has ended, the larvæ one by one swim upward until all are in active swimming movement. This activity continues for about 30 to 40 seconds, then the larvæ cease swimming one by one and settle to the bottom. If now the vial is covered by a jacketed tumbler or is otherwise thrown into a relatively deep shadow, the response of every larva is immediate and vigorous. There is no latent period. They swim actively to the top of the vial and continue swimming for an interval perhaps slightly longer than is the case when activated by an increase in light intensity, then they again cease activity one by one and sink to the bottom. It is as if a chemical reaction involving an explosive liberation of energy occurs at the instant a sudden shift is made from light of a higher to a lower intensity and as if a chemical change of a reverse kind takes place when the shift is from light of lower intensity to a higher, the latent period indicating an energy absorbing process. The substance in which these reversible reactions take place is probably photochemical in nature and located in the pigmented light-sensitive sense organ. It probably has no relation to the mechanism of metamorphosis, but in the early part of the investigation it was assumed to be directly connected with this mechanism. This substance and the reactions occurring in it, due to an effect of light, give origin to nerve impulses to the central nervous system in response to which the latter originates and transmits the impulses to the muscle bands by which swimming activity is initiated and controlled. Only secondarily is it causally connected with the origin of the by-products of neuromuscular metabolism

which now are assumed to constitute an essential part of the mechanism of metamorphosis.

Observation of larval responses to stimulation provided the final evidence by which the error to which reference has just been made was corrected and by which the facts were established that the process of metamorphosis is accelerated by a reaction involving a chemical by-product of larval activity and not as a direct effect of experimental treatment with light intensity changes, mechanical jarring or agitation. Several groups of larvæ were closely observed during treatment by the many methods devised, and it was noted that all larvæ are thrown into vigorous swimming movement at each light-intensity change, but that they often failed to be activated by either form of mechanical treatment although some activity was thus induced. It was then found that usually the percentage of metamorphosis that follows corresponding amounts of the several methods of treatment tends to be proportional to the amount of swimming activity induced by the treatment and not to the amount or duration of treatment, the effectiveness of activity depending upon the existence at the time of treatment of the susceptibility factor elsewhere discussed.

Observation of larval responses also provided the answer to the question why light-intensity changes made at intervals of one minute or longer are more effective in inducing activity, and hence metamorphosis, than similar treatment in which the changes are made at intervals of 30 seconds or less. All larvæ are thrown into activity at each such stimulation when the interval is one minute or longer, but stimulation made at the shorter intervals often fails to induce swimming. The probable reason for this difference in effect is that the shorter interval does not give time for the neuromuscular mechanism of the larvæ to recover from a condition of fatigue due to the previous burst of swimming activity, and that until the condition of fatigue has passed it serves as a block to further stimulation.

THE ASCIDIAN LARVA A DUAL ORGANISM

In the larva of ascidians two quite distinct structural systems are present, a larval action system and a more or less well-differentiated action system of the ascidiozooid. Larval action systems, as developed in three types of ascidian larvæ, have been fully described (Grave, 1921, 1926 and 1932). That of the *Botryllus* type, with which this study is chiefly concerned, consists of a central nervous system, three sense organs (light sensitive, static and probably tactile, the latter connected with a peripheral nerve plexus and group of ganglia), a notocord traversing the entire axis of the tail and two muscle bands closely applied to opposite sides of the notocord. The part of the mantle covering the body of the larva belongs to both larva and ascidiozooid and is the only part of the larval action system not destroyed at metamorphosis. The action system of the developing ascidiozooid consists of oral and atrial siphon, an alimentary canal, a ganglion,

hypophysial duct, subneural gland, pericardium and heart. The extent to which these structures are differentiated during the free-swimming period of the larva differs greatly in larvæ of different species; extremely rudimentary in some (*Molgula citrina, Polyandrocarpa tincta*), almost ready to function in others (*Perophora viridis, Ecteinascidia turbinata*).

Both action systems originate and continue to develop together for a short time, but as the larval action system becomes fully differentiated it completely dominates and inhibits the further growth and differentiation of the action system of the ascidiozooid. This inhibition continues until the larval action system is disrupted at metamorphosis even when the free-swimming period has a duration of five days or longer. Practically no change in the size, form or structure of a larva takes place from the time of its hatching or liberation until metamorphosis.

Immediately after metamorphosis occurs, growth and development of the ascidiozooid is resumed and continues with the same exceeding rapidity that characterizes the development of the larva.

The ascidian larva is thus a dual organism, the life of the larva incompatible with that of the ascidiozooid. Before the ascidiozooid may take the form and function of the sessile ascidian, larval form and function must be disrupted and destroyed.

It is fascinating to speculate on its inherent possibilities. The ascidian larva may be regarded as having been evolved as an adaptation of the floating ascidian egg and embryo to pelagic life. Here may be the origin of the organization peculiar to the vertebrate subphylum. Here the respiratory pharnyx of the ancient invertebrate stock of the ascidian was combined with the cœnogenetic larval notocord and it axial musculature and hollow axial nerve cord. It is possible to see in appendicularia a sexually mature ascidian larvæ in which metamorphosis and development of the sessile ascidian has been completely suppressed. The ancestral vertebrate line may be another that has originated in the same way. In any case, one can not escape the conviction that in ascidian metamorphosis, a higher organization with great possibilities is sacrificed to a lower.

THE DISRUPTIVE PHASE OF METAMORPHOSIS

The violent closing phase of metamorphosis during which the larval action system is disrupted usually occupies a very short period of time, usually not longer than about seven to twelve minutes, but its duration varies even in the same species. It is most rapid in larvæ that transform after a relatively short free-swimming period, slower in old larvæ that have about exhausted their reserve supply of energy by long-continued swimming. When long delayed, metamorphosis is frequently not preceded by attachment of the larva and the axial structures of the tail may not be completely withdrawn, a portion protruding temporarily or

permanently from the posterior end of the ascidiozooid. The sequence and character of its events may be observed with a watchmaker's lens.

A larva that is about to metamorphose develops a negative response to light and a positive response to gravity. On its final downward swim it attaches to the side or bottom of the vial. For a few moments after attachment, the tail continues to vibrate but with rapidly diminishing rate and amplitude. It now becomes rigid in what appears to be a tetanus-like tremor during which it slowly sways first to one side, then to another, as if under high tension from strong simultaneous contractions of both muscle bands. This tension continues and increases until the notocord, to which the muscle bands are terminally attached, gives way, bends, buckles and, with the entire tail mechanism, slowly slips backward within the noncellular tunic-sheath of the tail and is carried into the posterior portion of the body of the larva by the final contraction of the muscle bands and elasticity of the mantle.

Steps in this process in the larva of *Phallusia nigra* are shown in figures 5 to 9 of Plate 2. The notocord and its adhering structures, as they enter the body take the form of a tight but regular coil. By the second day after metamorphosis the tissues of the coil have disintegrated into an amorphous mass of cellular débris, and usually by the third day this has been largely absorbed, probably by phagocytosis.

These observations and others to be discussed in another part of the report may be interpreted as indicating that the initial event in metamorphosis should be ascribed to a condition of increased excitability of the neuro-muscular mechanism of the larval action system, probably induced by the accumulation in these tissues of a substance formed by a reaction between the "susceptibility" substance, elsewhere described, and some product of larval activity.

Berrill (1929, page 61) states that the disruptive phase of metamorphosis in the larva of *Phallusia mammillata* is initiated by phagocytosis of the tail, this being primarily conditioned by an increasing acidity of the tissues concerned, brought about by the activity of the tadpole, possibly through the accumulation of lactic acid.

My observations do not lead me to believe that phagocytosis plays any rôle in the initiation of the disruptive phase of metamorphosis, but that the function of phagocytosis is confined to the disintegration and absorption of the disrupted larval action system.

VARIABILITY OF LARVÆ

The larvæ of a species of ascidian are remarkably uniform in size, form and structural organization but show extreme variability in certain of their physiological characters; the duration of the free-swimming period and the duration of the interval between hatching from the egg or liberation by the brooding parent and the appearance of a reversal of orientations to light

and gravity during swimming activity. Larvæ of all species that have been the subject of behavior studies show initially, and for a variable interval of time, a negative orientation of swimming movements to gravity and, those that possess a light receptor, a positive orientation to light. Variability in these characters seems to be as marked in the individuals of a brood of the same age and parentage as among individuals of different parentage. Its nature and source are problems requiring consideration for it enters as an important factor in evaluating the results of all attempts to analyze the processes and mechanism of metamorphosis.

Are these variations to be accounted for on the basis of differences in genetic constitution of larvæ or are they due to differences in some environmental condition to which larvæ are exposed during the developmental or free-swimming periods? Whether this striking variability is due in considerable part to an effect of the unnatural monotony of the laboratory environment with its lack of movement of the water and the uniformity of light and pressure conditions, is a question difficult to determine because of the impossibility of observing an organism so small and active in its natural habitat, but it may be greatly accentuated by these unnatural laboratory conditions.

It has been found, by varying certain environmental factors, that the free-swimming periods of larvæ may be greatly shortened but the individual variations can not be thus wholly eliminated or obliterated. They still persist and take the form of a skew curve of frequency when plotted. Any form of stimulation of groups of larvæ that consistently excites them to swimming activity may shorten the free-swimming period; for example, changes in light intensity or mechanical agitations or jarrings made at frequent intervals, but this result is not produced in a larva until it has undergone some unknown internal change, probably correlated normally with ageing or the differentiation of some larval organ. Until this change has occurred, activity has no effect in shortening the free-swimming period. Larvæ which have developed this condition may be designated as "susceptible" and that which has rendered them susceptible to the accelerating effect of activity as the "susceptibility factor."

The variability of larvæ in the duration of the free-swimming period apparently depends upon and is an expression of the variability of the susceptibility factor in the time of its appearance. Its development or differentiation in larvæ of the *Botryllus* type is in no way influenced by light or mechanical stimuli or by any by-product of the swimming activity induced by such stimuli, but certain observations of larvæ of *Phallusia nigra* indicate that differentiation of the susceptibility factor in larvæ of this species is greatly accelerated by crowding. When, shortly after hatching, a thousand or more *Phallusia* larvæ are placed in a shell vial containing about 10 cc. of sea-water, they pass rapidly through the sequences of their orientations to light and gravity, and metamorphosis occurs in all of the larvæ in about

two hours, but if two or three hundred larvæ of the same lot are placed in a beaker containing 500 cc. of sea-water, some of the larvæ may metamorphose during the first 8 or 10 hours, but many of them for a period as long as five days will continue to swim when stimulated, and to manifest the initial positive response to light and negative response to gravity.

Berrill (1929) discusses variability of larvæ and ascribes the great differences in individuals of the same lot to the influence of two variable factors: the length of time an egg remains in the oviduct and the physiological condition of the parent during that time. What length of time he considers short or long is not definitely stated, but it is assumed by Berrill that in *Phallusia mammillata* the eggs may take several weeks or possibly months to pass through the oviduct, thus giving ample time for the oviducal fluid to influence the egg.

This explanation of variability of larvæ of *P. mammillata* is obviously not valid for that of larvæ of *Symplegma* or *Polyandrocarpa*, for in these species the same parent colonies produce broods of larvæ day after day in rapid succession throughout the summer, indicating rather conclusively that the entire formation and development of the eggs must take place in a relatively short time. The length of time the eggs of *Phallusia nigra* remain in the oviduct can not be so certainly estimated, since it is necessary to sacrifice the adult animal to get a lot of eggs, but certain observations indicate that eggs can not be so retained longer than about three weeks. Once during each lunar month at the Tortugas there is a period of nearly a week when it has been difficult to get eggs from *Phallusia* in sufficient number for experimental work, the oviducts of practically all adult individuals being empty at this time, but within a few days the oviducts are again filled to bulging, indicating that egg production is not a long process. In *Phallusia*, as in *Polyandrocarpa*, egg production is probably rapid and continuous.

Whether the physiological condition of the parent ascidian varies sufficiently during egg production to account for larval variability is a question for which no conclusive answer can be given, but in the thriving colonies of *Polyandrocarpa* that produced the extremely variable larvæ used in experiments, elsewhere described in this paper, it seems improbable. The explanation of larval variability to which my experience tends is that it is closely correlated with variability in the differentiation and functional activity of some larval organ or tissue. Some evidence was found in the part of this investigation carried out in 1933, in which larvæ were subjected to various substances added to sea-water, that differentiation of the susceptibility factor was either accelerated or compensated for by sea-water media containing KOH, NaOH and certain ascidian tissue extracts. It is possible, however, that the broods of larvæ used in experiments with these media, like many of those with which the earlier work was done, all hap-

pened to belong to broods in which a condition of susceptibility developed almost immediately after liberation.

ACCELERATION OF METAMORPHOSIS BY LIGHT

That light normally plays an important rôle in conditioning larvæ for the process of metamorphosis was first indicated by the observations made during the summer of 1927, an account of which is given in the introduction to this report (page 211), and although it has been necessary to change the interpretation then made of the manner in which light operates to influence the chain of events that constitutes metamorphosis, the fact remains that stimulation by light is by far the most effective physical method yet devised of inducing an epidemic of metamorphosis in a group of susceptible larvæ, hours in advance of a single instance in a group not so stimulated. The structural organization of ascidian larvæ, with its extremely sensitive light-perceiving sense organ, indicates that light is the form of energy that in nature supplies the stimuli by which the swimming movements of larvæ are activated and oriented.

The methods of treatment of larvæ with light and some of the experiments in which they were used, will be described, not as methods of reaching directly and activating the mechanism of metamorphosis, as was believed at the time the work was done, but as evidence of the effectiveness of light as a means of stimulating larvæ to the swimming activity necessary for the production of the metabolic by-product by which the process of metamorphosis in susceptible larvæ is induced.

Prompted by the intimation that light may produce effects that induce metamorphosis, a program of experimentation began in 1927, having for its object the development of a method of treating groups of larvæ with sunlight, in the hope that means would be found by which metamorphosis could be induced at will. Twenty-three experiments were carried out during the two weeks that remained of the season of 1927, some of which were sufficiently successful to encourage the belief that progress in the desired direction was being made. In all of these experiments, larvæ segregated in 50 cc. beakers were exposed to direct sunlight for periods of time varying in number and duration, after which the beakers were placed in very diffuse light or darkness, except for brief intervals when removed for examination. An outline of one of the most successful of these experiments, made on July 24, follows:

8:00- 8:10 a.m.	78 *Symplegma* larvæ collected as liberated and placed in a 50 cc. beaker (diffuse light of laboratory).
8:10- 8:15	Exposed 5 minutes to direct sunlight.
8:15- 8:17	Beaker covered 2 minutes with jacket of black paper.
8:17- 8:48	Beaker with jacket removed, placed in an opaque container, covered with black cloth, 31 minutes.
8:48- 8:58	Beaker exposed 10 minutes to direct sunlight.

8:58- 9:05	Beaker in same cloth-covered container.
9:05	Beaker examined in diffuse light. *One larva metamorphosed.*
9:05- 9:15	Returned to cloth-covered container.
9:15	Examination. *3 additional larvæ metamorphosed.*
9:15- 9:45	Returned to cloth-covered container.
9:45	Examination. *17 additional larvæ metamorphosed.*
9:45-10:20	Returned to cloth-covered container.
10:20	Examined. *24 additional larvæ metamorphosed.*
10:20-11:25	Returned to cloth-covered container.
11:25	Examination. *17 additional larvæ metamorphosed.*

This result (78 per cent metamorphosis of the group of treated larvæ) is to be compared with that obtained with another group of 26 larvæ of the same parentage that may be considered a control. The latter group, ten minutes after liberation at 8:30 a.m., was exposed 30 seconds to direct sunlight, then covered with a black paper jacket 1 minute, and then placed in diffuse light until 11:30 a.m. Examination at this time showed one larva only had metamorphosed.

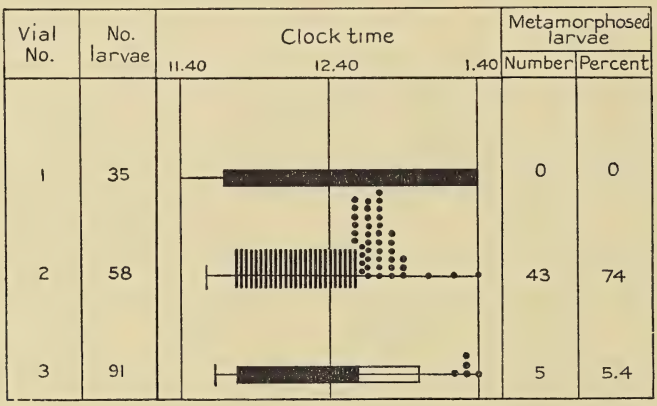
In accordance with the implications of the tentative hypothesis that metamorphosis is induced by exposures to sunlight, the work planned for the season of 1928 had for one of its aims a precise determination of the quality of light required to induce metamorphosis: whether by white light or by one of its component wave-lengths and whether ultra-violet rays may be involved in the reaction. By the use of a beam of sunlight reflected downward into the water from the silver mirrors of a heliostat it was sought to avoid changes in the composition of light due to its passage through the glass wall of vessels containing larvæ. For the exclusion of ultra-violet rays, two thicknesses of optical glass for covering the tops of containers during experimentation were provided, and it was proposed to expose larvæ to light of various wave-lengths by the use of a set of Wratten light filters. Related questions proposed for study were whether larvæ undergo a process of ageing or preparation in the interval between liberation and metamorphosis; whether ageing occurs in the absence of light or at low temperatures; whether larvæ are active at low temperatures and whether their activity plays a part in conditioning them for metamorphosis.

Large colonies of *Symplegma viride* could not be found during the early part of the season of 1928, and after some delay several colonies of *Polyandrocarpa tincta* were substituted. The larva of this species is the same in structural type as that of *S. viride* only smaller. It proved to be even better adapted to experimentation and has been available for all subsequent work.

As the planned program of work with this larva progressed, it soon became apparent that the mechanism of metamorphosis and light have no specific "lock and key" relationship, hence it would be more than useless to burden this narrative with complete descriptions of the installation of a

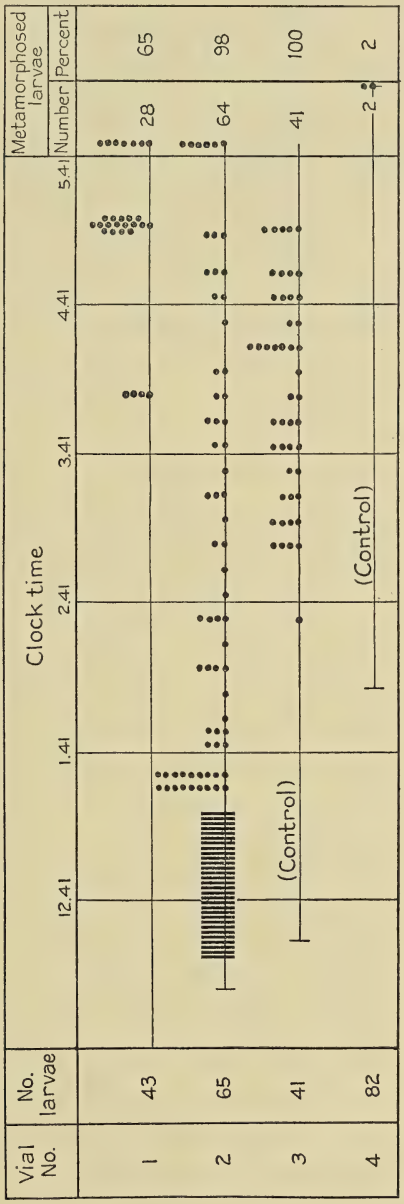
heliostat and accessory apparatus necessary for the use of Wratten filters, this work being discontinued when it became clear that the solution of the problem of the acceleration of metamorphosis lies in a direction that does not involve quantitative investigations of light. It is referred to merely as a matter of record. Two statements, however, concerning results of this work may be made; ultra-violet rays were found to play no part in accelerating the process of metamorphosis, and no particular wave-length of visible light was found to have more effect than another; that is, larvæ were stimulated to swimming activity by intensity changes of light from which the ultra-violet rays had been removed, and visible light of any wave-length seemed to be as effective in activating larvæ as white light.

It was found by empirical methods that sunlight, to be most effective in inducing or accelerating metamorphosis, must be delivered, not in the form of a continuous beam, but in a series of discrete doses separated by brief intervals of shadow or darkness. When the doses or intervals of light to which groups of larvæ were exposed had a duration of about 1 minute and were separated by an interval of darkness or shadow of similar duration, a maximum effect seemed to be obtained. Progressively smaller percentages of metamorphosis occurred when the intervals of alternate light and shadow were shorter than 1 minute. Momentary interruptions of a beam of sunlight, produced one per second by a horizontal bar attached to a pendulum swinging between the vial and the heliostat, had no effect whatever. Larvæ so treated throughout a period of four hours showed no tendency to meta-



Graph 1: Experiment 51, July 30, 1928. Larvæ (*P. tincta*) collected between 11:45 and 11:57 a.m. in three vials (5 x 1.8 cm.). Vial 1 placed in a dark-chamber: vial 2 in cooling vessel in "exposure booth" from 12:00 to 12:50 p.m. and given 25 1-minute exposures to a heliostat beam of sun-light each followed by a 1-minute exposure to very diffuse light (Heliostat beam interrupted by a 10 x 12 plate holder cover) then removed to a laboratory table; vial 3 placed in a dark chamber until 12:51 then to the cooling vessel in the exposure booth and given a 25-minute exposure to the heliostat beam of sun-light, then removed to the diffuse light of the water table. Examinations were made of vials 2 and 3 at intervals of 5, then 10 minutes until 1:40 p.m. when the experiment ended. One examination of vial 1 at 1:40 p.m. Each black circle above the horizontal lines indicates approximate time of metamorphosis of a larva.

morphose and continued free-swimming life as long as the untreated controls. It was as if the beam of light were continuous.



GRAPH 2: EXPERIMENT 52, August 1, 1928. Larvæ (*P. tincta*) liberated 11:41 to 12:24 p.m., collected in four vials (5 x 1.8 cm.). Vial 1 placed in cooled dark-chamber: vials 2 and 3 on a laboratory table: vial 4 in the water-table immersed to depth of 1.5 cm. (very diffuse light). From 12:17 to 1:17 p.m. larvæ in vial 2 given 30 1-minute exposures to darkness each followed by a 1-minute exposure to the diffuse sun-light of the laboratory. Examinations of vials 2 and 3 made at 5- and 10-minute intervals throughout afternoon; of vial 1 at 4:04, 5:10 and 5:48 p.m.; of vial 4 at 6:07 p.m. Each black circle above the horizontal lines indicates approximate time a larva metamorphosed.

Results of three selected experiments made during this period and demonstrating the effectiveness of this method of treatment are shown in Graphs 1, 2 and 3. An epidemic of metamorphosis usually occurred immediately

after the completion of treatment consisting of 20 to 30 one-minute exposures to light, each followed by a like interval of shadow or darkness.

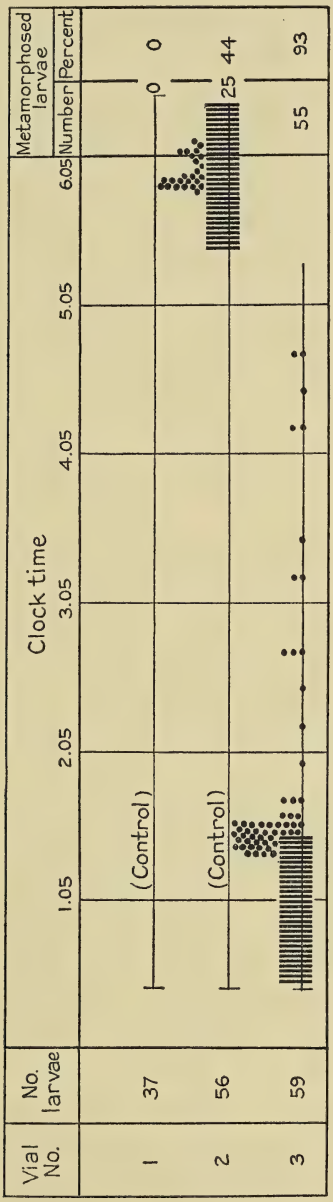
By reference to Graph 2, in which the data of experiment 52 are shown, it may be noted that numerous cases of metamorphosis occurred in the untreated control lots of larvæ (in vials 1 and 3) as well as in the treated larvæ in vial 2, but with the difference that they occurred much later in the untreated larvæ. These unexpected and disconcerting cases of metamorphosis that were taking place at this time with more or less frequency in the control vials of all experiments were calling for some explanation. They were not in harmony with the working hypothesis that light is the effective agent in causing metamorphosis in proportion to its intensity and quantity.

The vials containing control lots of larvæ for experiments during this period were kept in a very poorly illuminated receptacle in the Mills Water Table on the assumption that they should be shielded as much as possible from the effect of light. They were removed occasionally for examination to a much better lighted laboratory table, then returned to the dimly lighted receptacle.

The required explanation for these results seemed to be provided by the assumption that the effective parts of the treatment inducing or accelerating metamorphosis in these larvæ, both treated and control lots, are the sudden changes in light intensity occurring during an experiment rather than an effect of light energy as such; and that the effect produced is not proportional to the intensity or quantity of the beam of light that is periodically interrupted, but is the same for all intensities. Thus the cases of metamorphosis that occurred late but with increasing frequency in the control vials might be accounted for as due to the changes in light-intensity that took place twice during each examination; once when the vials were removed from the dimly lighted receptacle to a well-illuminated table, again when they were returned to the receptacle.

When this modified hypothesis was put to the test of experiment near the close of work in 1928, the clean-cut results obtained seemed to prove its validity. The result of the first of these experiments, Number 53, is shown in Graph 3 and is fully described in the text, as follows: A brood of *Polyandrocarpa* larvæ liberated between 12.05 and 12.30 p.m., was collected with a pipette and placed in three shell vials 1.8 cm. in diameter and 5 cm. in height, each containing sea-water to a depth of 4.5 cm. These vials were placed about 1 meter apart on a table located in the interior of the large laboratory about 2½ meters from a row of east windows from which the room was lighted during the experiment. All other sources of light were closed. During the experiment, rain clouds covered the sky, reducing the diffuse light to an unusually low intensity. Two of the vials, containing 56 and 37 larvæ, respectively, constituted controls for the experiment. Great care was taken that no change in the intensity of the light to which these controls were exposed should occur at any time. During examinations

that were occasionally made of these vials during the afternoon, they were not touched or in any way shadowed. They stood in light of uniform intensity throughout the experiment. The vial containing 59 larvæ was given



GRAPH 3: EXPERIMENT 53, August 3, 1928. Larvæ (*P. tincta*) collected between 12:05 and 12:33 p.m. distributed to 3 vials (5 x 1.8 cm.). Vials placed in similar positions with reference to light one meter apart on a laboratory table. Larvæ in vial 3 given 30 1-minute exposures to darkness each followed by a 1-minute exposure to diffuse sun-light. Examinations of vials 2 and 3 made at intervals of 15 minutes without touching or shadowing the vials; of vial 1 at 5:20 and 6:30 p.m. Black circles above horizontal lines indicate metamorphosed larvæ. Between 5:26 and 6:26 p.m. larvæ in vial 2 given same treatment previously given vial 3, examinations being made during each exposure to light without touching or shadowing the vial.

a treatment consisting of 30 one-minute exposures to the diffuse light of the room, each followed by a one-minute exposure to darkness, produced by covering the vial with a tumbler jacketed with black paper, the entire

treatment extending from 12:33 to 1:33 p.m. Attachment and metamorphosis of the treated larvæ began at the conclusion of the 25th exposure, and 28 larvæ had attached and metamorphosed within one minute after the 30th exposure. 18 of the remaining larvæ attached and metamorphosed, one by one, within 3 hours after the treatment ended, as the vial stood in diffuse light of uniform intensity. Three larvæ did not metamorphose and they died the following day.

None of the larvæ in the control vials had metamorphosed 5 hours after their liberation. At this time, 5:26 p.m., the control vial containing 56 larvæ was given the same treatment to which the experimental vial had been subjected, with the result that these larvæ began to attach and metamorphose at the 13th exposure to light; 1 at the 13th, 6 at the 14th, 5 at the 15th, 3 at the 16th, 1 each at the 17th, 18th and 19th, 2 at the 20th, 3 at the 21st and 1 each at the 22d and 23d. The remaining 31 larvæ retained the larval form until they died the following day. None of the 37 larvæ of the other control had metamorphosed at sunset (7:15 p.m.), more than 6.5 hours after their liberation.

The very great success of this method of treatment of groups of larvæ raised the question, how does it produce its accelerating effect on the processes of metamorphosis?

If the specific agency by which the observed acceleration of metamorphosis is induced, is not the light energy transmitted to larvæ so treated, is it to be found in the series of "shocks" that may occur at the *makes* and *breaks* in the flow of light energy, which may be conceived as capable of disturbing a delicately balanced internal equilibrium, thus setting in motion the chain of metabolic events that constitute the mechanism of metamorphosis?

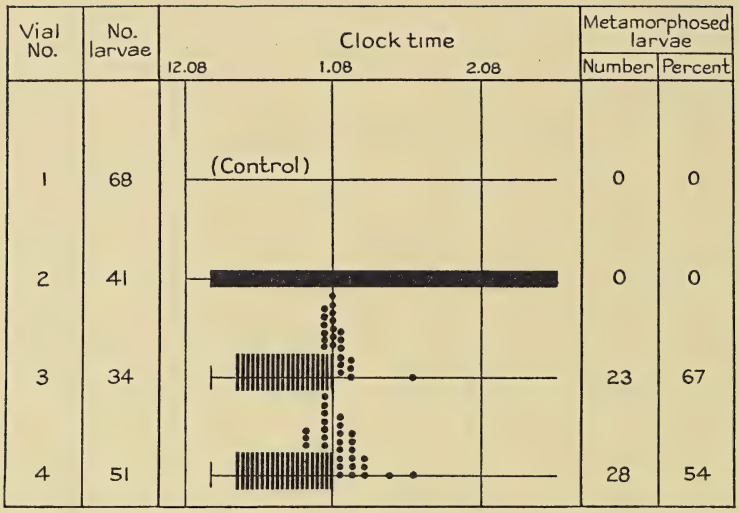
Certain aspects of the behavior of larvæ while under experimental treatment, fully described on page 222, seemed to lend support to such an interpretation of the nature of the effect of this method of treatment; namely, the explosive aspect of the response all larvæ make at the instant a reduction of light intensity is made; as if, at this instant energy were suddenly released thus stimulating the larva vigorously. The latent period that follows each sudden increase in light intensity, during which larvæ remain motionless for 3 to 10 seconds, may indicate the reverse chemical reaction during which energy is absorbed.

Such an interpretation, carried to its logical conclusion, was seen to involve the further suggestion and assumption that "shocks" of similar nature and import may be produced at the moment of any sudden stimulation: by repeated sudden mechanical or electrical stimulations or sudden changes in temperature and that they may be as effective in starting the process of metamorphosis as shocks due to sudden changes in light intensity. Experiments were planned for the summer of 1929 to test the validity

of these suggestions. Those that involved the application of mechanical methods of treatment of larvæ are described under the sub-title, "Acceleration by Mechanical Stimuli." Further work with light was also planned to ascertain how slight the changes in light-intensity levels may be and still be effective in inducing metamorphosis, and to compare the relative effectiveness of *slight* and *wide* changes in the levels of light-intensity. For this part of the program the equipment provided included: low-voltage electric light bulbs, mazda 1129, 21c and 50c; storage batteries; voltmeter and resistance coil by which to maintain a constant current of 5 volts across the lamps; a heliostat by which a vertical beam of sunlight of very high candle power could be used simultaneously with light from the electric bulbs; a light-proof exposure tunnel and box in which larvæ could be exposed to light from the electric bulbs at distances of 23.5 cm., 45.8 cm. and 90 cm.; a platinum backed mirror for reflecting the beam downward in a vial containing larvæ, and an electric fan for drawing the heat produced by the electric bulbs from the exposure tunnel.

Before many experiments had been made in 1929 with this equipment, the positive results obtained from mechanical treatment of larvæ had made it apparent that the tests proposed can contribute nothing pertinent to an analysis of the process and mechanism of metamorphosis. This experimentation was therefore discontinued, but some of the results may be of interest in that they indicate the very low threshold of the light-perceiving sense organ of the ascidian larva to stimulation by light and show the slight differences between two levels of light-intensity required to stimulate larvæ to vigorous activity; series of one-minute exposures to a beam of light from a 32c electric bulb, each followed by a one-minute interval of darkness, were consistently less effective in inducing metamorphosis than corresponding series in which similar alternate exposures were made between the diffuse sun-light of the laboratory and darkness, but the effectiveness of the latter was found to be about equal to that of corresponding series in which a 50c bulb was substituted for the 32c bulb. (Experiment 77, Graph 4.)

The narrative thus far has had reference to methods and results of work with larvæ of *Symplegma viride*, *Polyandrocarpa tincta* and *P. gravei*, all having the *Botryllus* type of structural organization, but when the methods of treatment that were so effective in inducing a high percentage of metamorphosis in groups of larvæ of the *Botryllus* type were applied to groups of larvæ of a widely different structural type, *Phallusia nigra*, the percentage of metamorphosis induced was consistently too small and variable to be considered significant. Results of three experiments (93, 96 and 101), in which groups of *Phallusia* larvæ were subjected to changes in light intensity made at intervals of 1 minute, in series varying in number between 60 and 150, are shown in parts of tables 1, 2 and 3. The percentages of meta-



GRAPH 4: EXPERIMENT 77, August 9, 1929. Larvæ (*P. tincta*) liberated between 12:08 and 12:28 p.m., collected in 4 vials (5 x 1.8 cm.). Vial 1 placed on laboratory table in diffuse sun-light: vial 2, in water-table in darkness (vial covered with tumbler jacketed with black paper); vial 3 placed in light-proof exposure box and given 20 1-minute exposures to a beam of light from a 50c auto head-light bulb 23.5 cm. distant, each followed by a 1-minute exposure to darkness, from 12:28 to 1:08 p.m.; vial 4, on laboratory table near vial 1, given 20 1-minute exposures to darkness (vial covered with tumbler jacketed with black paper), each followed by a 1-minute exposure to the diffuse sun-light of the laboratory. Examinations of vial 1 made at 2:00 and at 2:30 p.m.: vial 2 at the end of the experiment only; vial 3 at 1:08 p.m. and thereafter at intervals of 5 minutes: vial 4 examined during each exposure to light and thereafter at intervals of 5 minutes. Each black circle indicates the time of metamorphosis of a larva.

morphosis thus induced varies from less than that which occurred in the untreated controls to about five times that in controls, and is never high.

The lack of effectiveness of this method of treatment is not due to any lack of response of *Phallusia* larvæ to stimulation by light intensity changes, for this larva is extremely sensitive to passing shadows, but it is apparently due to the failure of the method to establish the specific rhythm of stimulation and response to which this larva is innately tuned. If groups of this larva are placed under conditions in which they are free to determine their own rhythm of response to light stimulation, they usually show a consistently high percentage of metamorphosis.

These conditions are provided by the use of a shell vial container about the bottom end of which is wrapped a band of black paper one to two centimeters in width. The size of the vials that have been successfully used vary in width from 1.8 to 2.2 cm. and from 5 to 8 cm. in height. Vials of larger diameter do not provide the conditions required. By placing vials so prepared on the black paper covering of the Mills Water Table, the bottom part of each is converted into a dark chamber, the upper part being relatively well lighted.

TABLE 1—*Experiment No. 93: 7, 4, '30. Phallusia nigra. Eggs fertilized 5:55 a. m. Hatching at 2 p. m. Vials stocked at 2:20 p. m. Formalin added at 6:10 p. m.*

Vial number	Size of vial	Volume of water	Age of larvæ	Number of larvæ	Kind of treatment	Duration of treatment	Metamorphosed larvæ	
							Number	Per cent
1	5 × 2.2 cm.	13 cc.	25 min.	250	Control.	4 hrs., 10 min.	8	3.2
2	"	"	"	179	75 1-min. exposures to diffuse light, each followed by a 1-min. exposure to darkness—then a second set of 15 1-min. exposures to light followed by 1-min. exposure to dark.	2½ hrs. (2:25–4:55) and ½ hr. (5:35–6:05).	5	2.7
3	"	"	"	161	150 Agitations of the water once per minute, then a second set of 30 similar agitations.	"	9	5.5
4	"	"	"	247	Control.	4 hrs., 10 min.	1	.4
5	"	"	"	158	Band of black paper 1.8 cm. wide around bottom end of vial.	"	82	51.9
6	8 × 2.2 cm.	25 cc.	"	174	Control.	"	1	.5
7	"	"	"	150	Band of black paper 1.8 cm. wide around bottom end of vial.	"	33	22.0

TABLE 2—*Experiment No. 96: 7, 7, '30. Phallusia nigra. Eggs fertilized 6 a. m., 7, 5, '30. Larvæ hatched 2 to 2:15 p. m. Kept in 500 cc. beaker in Mills Water Table. Vials stocked at 9:22 a. m., 7, 7.*

Vial number	Size of vial	Volume of water	Age of larvæ	Number of larvæ	Kind of treatment	Duration of treatment	Metamorphosed larvæ	
							Number	Per cent
1	5 × 2.2 cm.	13 cc.	31 hrs.	76	50 1-min. exposures to diffuse light each followed by a 1-min. exposure to darkness.	1 hr., 40 min. (9:25–11:05).	7	9.2
2	"	"	"	126	45 1-min. exposures to diffuse light each followed by a 1-min. exposure to darkness.	1 hr., 30 min. (9:25–10:55).	11	8.7
3	"	"	"	100	Control.	1 hr., 33 min. (9:22–10:55).	2	2
4	"	"	"	88	Band of black paper 1.8 cm. wide around bottom end of vial.	1 hr., 33 min. (9:22–10:55).	29	33
5	"	"	"	76	"	1 hr., 05 min. (9:22–10:25).	11	14

TABLE 3—*Experiment No. 101: 7, 12, '30. Phallusia nigra. Eggs fertilized at 5:30 a. m. Hatching at 1:10 p. m. Vials stocked at 1:40 p. m. Formalin added to all vials at 3:45 p. m.*

Vial number	Size of vial	Volume of water	Age of larvæ	Number of larvæ	Kind of treatment	Duration of treatment	Metamorphosed larvæ	
							Number	Per cent
1	6 × 1.8 cm.	9 cc.	1½ hrs.	62	30 1-min. exposures to diffuse light each followed by a 1-min. exposure to darkness.	1 hr. (2:45–3:45 p.m.).	8	12.9
2	5 × 2.2 cm.	13 cc.	"	130	"	"	8	6
3	6 × 1.8 cm.	9 cc.	30 min.	104	Control.	2 hrs., 5 min.	2	1.9
4	"	"	"	113	Band of black paper 1.5 cm. wide around bottom end of vial.	"	49	43.3
5	"	"	"	116	Band of black paper 1.8 cm. wide around bottom end of vial.	"	18	15.5
6	5 × 2.2 cm.	13 cc.	"	118	Band of black paper 1.8 cm. wide around bottom end of vial.	"	24	20
7	6 × 1.8 cm.	9 cc.	"	132	Band of black paper 2 cm. wide around bottom end of vial.	"	36	27
8	"	"	"	114	Band of black paper 3 cm. wide around bottom end of vial.	"	14	12

TABLE 4—*Experiment No. 122: 8, 1, '30. Phallusia nigra. Eggs fertilized 7, 31, at 7:05 a. m. Hatching at 2:40 p. m. Vials stocked, 8, 1, at 8:45 a. m. Larvæ kept in 500 cc. beaker after hatching until used. Formalin added at 10:45 a. m.*

Vial number	Size of vial	Volume of water	Age of larvæ	Number of larvæ	Kind of treatment	Duration of treatment	Metamorphosed larvæ	
							Number	Per cent
1	6 × 1.8 cm.	9 cc.	18 hrs.	106	Band of black paper 2 cm. wide around bottom end of vial.	2 hrs. (8:45–10:45).	51	48
2	"	"	"	91	"	"	41	48
3	"	"	"	90	"	"	40	45
4	"	"	"	83	Control.	"	10	12
5	"	"	"	79	"	"	11	13
6	"	"	"	99	"	"	14	14

Larvæ placed in such a vial may be seen to be continuously active, milling up and down between the well-illuminated upper part and the darkened chamber below, whereas in a vial without the band of black paper, swimming movements become gradually diminished and all larvæ sooner or later become relatively quiescent and finally lie motionless on the bottom. The continuous up and down movements of these larvæ may be interpreted as due to a rhythmic series of stimulations by the light-intensity changes produced at the moments the larvæ pass upward from the dark to the illuminated zone and vice versa.

The relatively high effectiveness of this method of treatment of *Phallusia* larvæ that have become "susceptible" may be seen by reference to vials 5 and 7 in table 1; vials 4 and 5 in table 2 and vials 4 to 8 in table 3. Experiment 122, summarized in table 4, in which *Phallusia* larvæ were subjected, 18 hours after hatching, to this method of treatment for two hours only, shows its remarkable effectiveness in inducing a consistently high percentage of metamorphosis.

Contrary to expectations, this method of treatment, however modified, is not effective in inducing metamorphosis in groups of larvæ of the *Botryllus* type as is shown by experiment 97, summarized in table 5, which is one of several in which groups of *Polyandrocarpa* larvæ were simultaneously subjected to both methods of causing changes in light intensity. These larvæ are not stimulated to continuous activity by the conditions provided by banded vials.

ACCELERATION BY MECHANICAL STIMULI

Observations made during the summer of 1928, previously referred to, led to the assumption that any method of treatment of larvæ that produces internal "shock" will accelerate the process of metamorphosis, and that shock due to mechanical, electrical or temperature stimulation may be as effective as treatment with sudden changes in light intensity. Accordingly, in 1929 an experiment for subjecting larvæ to a series of mechanical jars was devised, consisting of a small electric call-bell-apparatus from which the bell was removed and replaced by a small wooden shelf on which the vials containing larvæ were fastened. This shelf and the vials resting on it could be made to vibrate by impacts from the electrically operated bell clapper. The method of mechanical treatment with this apparatus to which larvæ were first subjected consisted in exposing them to 30 one-minute intervals of jarring alternating with one-minute intervals of rest; but later the series consisted of 60 ten-second intervals of jarring, each followed by a fifty-second interval of rest. Larvæ were mechanically treated also by agitating the water in the vials with a pipette once per minute for the duration of an experiment, usually one hour. At each agitation the pipette was slowly filled with water from the upper part of the vial, care being taken not to draw up the larvæ which were usually lying motionless on the bot-

TABLE 5—*Experiment No. 97: 7, 9, '30. Polyandrocarpa tincta, Larvæ liberated between 9:05 to 9:18 a. m. Seven vials stocked as indicated below.*

Vial number	Size of vial	Volume of water	Age of larvæ	Number of larvæ	Kind of treatment	Duration of Experiment	Metamorphosed larvæ	
							Number	Per cent
1	8 × 2.2 cm.	25 cc.	15 min.	47	60 1-min. exposures to diffuse light each followed by a 1-min. exposure to darkness (9:20–11:20), then after 2 hrs., 30 1-min. exposures to diffuse light each followed by a 1-min. exposure to darkness (1:20–2:20 p.m.). Formalin added at 2:20 p.m.	5 hrs., 15 min.	13	27.6
2	"	"	"	80			32	40
3	"	"	"	42	Control.	"	0	0
4	"	"	"	36	Band of black paper 0.5 cm. wide around bottom end of vial.	"	0	0
5	"	"	"	49	Band of black paper 1.5 cm. wide around bottom end of vial.	"	0	0
6	"	"	"	29	Band of black paper 2 cm. wide around bottom end of vial.	"	0	0
7	"	"	"	35	Band of black paper 3 cm. wide around bottom end of vial.	"	0	0

tom, then the pipette was slowly emptied in the vial with force just sufficient to carry all larvæ from the bottom.

In order that comparison might be made of the relative effectiveness of these mechanical treatments with that of other methods, each experiment included several vials stocked with larvæ of the same brood, one or more vials for each method of treatment, all treatments given simultaneously. No attempt was made in experiments of this kind to follow and record the cases of metamorphosis as they occurred in the several vials during treatment, but at the conclusion of a series of treatments all larvæ were killed by the addition of formalin to the vials. The metamorphosed ascidiozooids and free-swimming larvæ were then counted and the percentage of metamorphosis calculated.

The method of treatment of larvæ by mechanical jarring was employed in seven experiments in 1929, five in 1930. Treatments by agitation of the water with a pipette were made in nine experiments in 1930. The following tables show the results of these mechanical treatments as compared with corresponding treatment with changes in light intensity.

TABLE 6—*Results of treatment of Polyandrocarpa larvæ with a series of mechanical jarrings and a corresponding series of light-intensity changes.*

	Mechanical jarrings		Light-intensity changes		Controls	
Date	Number of larvæ	Per cent of metamorphosis	Number of larvæ	Per cent of metamorphosis	Number of larvæ	Per cent of metamorphosis
7-15-29	35	3	47	38	49	0
7-28-29	43	0	51	4	70	0
8-7-29	70	3	73	46	70	0
8-11-29	71	100	43	53	63	5
8-14-29	35	5	60	20	36	0
8-17-29	43	0	29	31	33	0
7-23-30	56	0	78	23	126	0
7-28-30	63	1.5	127	45	188	0
7-29-30	50	12	75	39	59	2.7
8-1-30	58	82	39	43	58	1.7
8-3-30	140	7	288	26	120	.8
Average		11		33		0.9

While the effectiveness of mechanical treatment in inducing metamorphosis was much less in all but two of the experiments than that of an equivalent treatment with light-intensity changes, metamorphosis resulted in a sufficiently large number of cases to demonstrate that the process requires no specific agent or form of stimulus to set it in motion and to give support to the assumption that any method of treatment of susceptible lar-

væ that is effective in stimulating them into swimming activity will induce it. Treatment of larvæ with repeated changes in light-intensity is more effective than other methods for the reason that ascidian larvæ have a sense organ that greatly lowers the threshold for stimulation by light, whereas jars and movement of the water often fail to stimulate larva into activity. The function of the light-sensitive sense-organ in stimulating larvæ to swimming movement probably depends upon chemical changes induced by light in a photosensitive substance in the "eye" but the evidence from ex-

TABLE 7—*Results of treatment of Polyandrocarpa larvæ with a series of agitations of larvæ with a pipette and corresponding series of light-intensity changes.*

	Mechanical agitations		Light-intensity changes		Controls	
Date	Number of larvæ	Per cent of metamorphosis	Number of larvæ	Per cent of metamorphosis	Number of larvæ	Per cent of metamorphosis
7-1-30	105	3	135	10	108	0
7-20-30	53	11	58	26	24	0
8-1-30	75	7	39	43	58	1.7
8-3-30	76	3	288	26	120	.8
Average		6		26		0.6

periments with mechanical methods points to larval activity itself as the source of the stimuli required to induce metamorphosis.

Attempts were also made during the summers of 1929 and 1930 to stimulate larvæ electrically, but without success. Small platinum electrodes were at first used, but later non-polarizable electrodes were set up and connected directly with the sea-water of a small vessel into which larvæ were placed. No difficulty was found in setting up electric currents of varying strengths in the water between the electrodes, but the larvæ in the water received no stimulus, because the conductivity of sea-water is higher than that of the body tissues of the larvæ. An organism as small as an ascidian larva can not be connected directly with electrodes, hence no further attempts to treat larvæ by an electrical method of stimulation were made. Later, when it became clear that it is not the direct effect of any agency on the mechanism of metamorphosis that induces or accelerates its process, but that the acceleration is due to some by-product of larval activity thereby promoted, the only remaining interest in the effect of electrical stimulation is whether an ascidian larvæ is capable of responding to such stimulation by swimming activity, and this may be taken for granted.

SUSCEPTIBILITY, ACTIVITY AND THE MECHANISM OF METAMORPHOSIS

After a large number of experiments had been made during the summer of 1929 in repeated but unsuccessful attempts to verify the results obtained in the closing days of the previous summer in which a high percentage of metamorphosis was induced in groups of larvæ by treatment consisting of 30 one-minute exposures to diffuse light, each followed by a one-minute exposure to darkness, it finally became apparent that the failures were not due to any fault in the method of experimental treatment, but to a complicating internal factor, the existence of which had not been suspected.

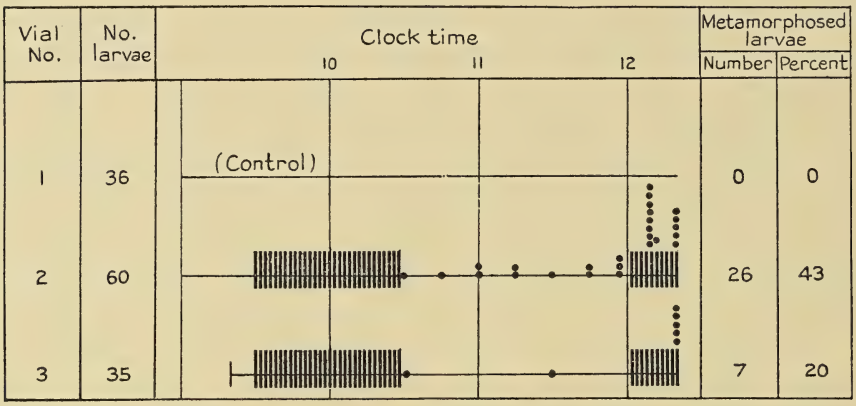
The numerous broods of larvæ with which these experiments dealt were produced throughout the summer by a colony of the same species that had supplied larvæ for the successful experiments with which the work of 1928 had ended, but the larvæ of these broods, with exceptions to which reference is made later, could not be induced to metamorphose until relatively long intervals of time had elapsed, varying from 3 to 8 hours after liberation, even though subjected to treatment involving as many as 150 and 180 changes in light intensity made at one-minute intervals. The same colony that liberated these "resistant" larvæ, however, produced two broods on July 8 and August 9, the larvæ of which were induced to metamorphose within an hour by the standard method of treatment, thus verifying the previous results to which reference has been made.

This very great variability and diversity of response to experimental treatment of larvæ of broods of the same parentage called for explanation which seemed to be provided by the assumption that some substance essential for metamorphosis, not present in larvæ at the time of their liberation, develops or differentiates during the free-swimming period; very early after liberation in some larvæ, relatively very late in others. Until this substance has appeared, a larva is not susceptible to the induction of metamorphosis, either naturally or experimentally. This hypothetical substance, because of its assumed function has been designated the "susceptibility" factor. Before its differentiation, larvæ are said to be "resistant." They lack something essential to the activation of the mechanism of metamorphosis.

Graphs of three of the 25 experiments made in 1929 with these diverse broods of larvæ are reproduced to show the extreme variability found in the time of differentiation of the susceptibility factor; one with a brood of larvæ in which it developed unusually early (Graph 4, Experiment 77); one in which its development was longest deferred (Graph 6, Experiment 81). An intermediate case is shown in Graph 5, Experiment 80.

An even greater variability in the time interval between liberation of larvæ of *Polyandrocarpa* and the appearance of the susceptibility factor was shown by broods obtained during the summer of 1930. This is shown in Graphs 7, 8, 9 and 10 which, respectively, present the data of experiments 111, 118, 121 and 123.

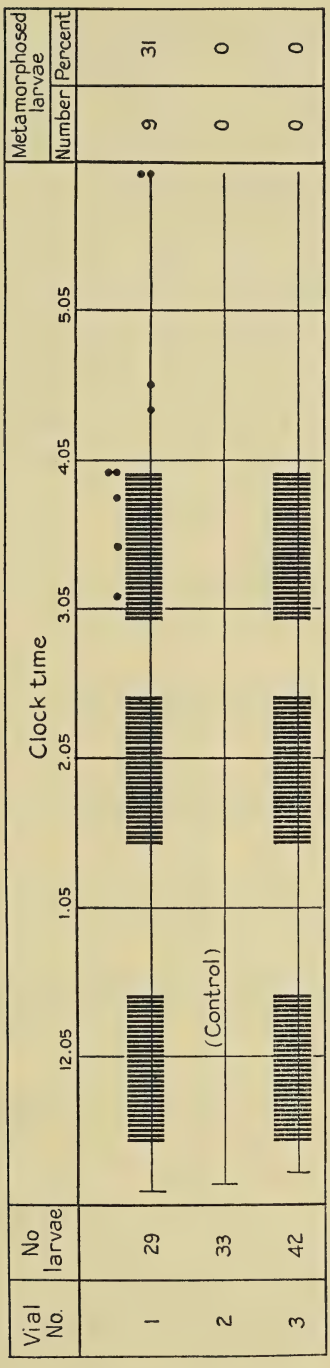
From some of these graphs it may be noted that, during the treatment of resistant larvæ, periods of "rest" for ageing of the larvæ were interpolated between the intervals of experimental stimulation, for it was apparent that continuous treatment did not hasten differentiation of the susceptibility factor.



GRAPH 5: EXPERIMENT 80, August 14, 1929. Larvæ (*P. tincta*) liberated between 9:10 and 9:25 a.m., collected in 3 vials (5 x 1.8 cm.). Vial 1 placed on laboratory table in diffuse sun-light of constant intensity; vial 2 on same laboratory table and given 30 1-minute exposures to darkness (covered by tumbler jacketed with black paper) each followed by a 1-minute exposure to diffuse sun-light (by lifting tumbler), from 9:27 to 10:27 a.m.; vial 3 placed on electric vibrator shelf and given 30 1-minute intervals of mechanical jarring each followed by a 1-minute interval of quiet, from 9:28 to 10:28 a.m. After an interval of 90 minutes during which treatment was discontinued, vials 2 and 3 were subjected to a repetition of the former treatment for 30 minutes. Examination of vial 1 was made at the conclusion of the experiment; of vial 2 at intervals of 15 minutes following the first treatment and at each exposure to light during the second treatment; of vial 3 at the same intervals and at the conclusion of the second treatment. Each black circle above the horizontal lines indicates the time of metamorphosis of a larva.

Of what this susceptibility factor consists, where and how it is produced, whether it is correlated with structural development or tissue differentiation, are questions to which no positive answer can yet be given but, as is later suggested on theoretical grounds, it is probably a secretory product of some larval organ or tissue.

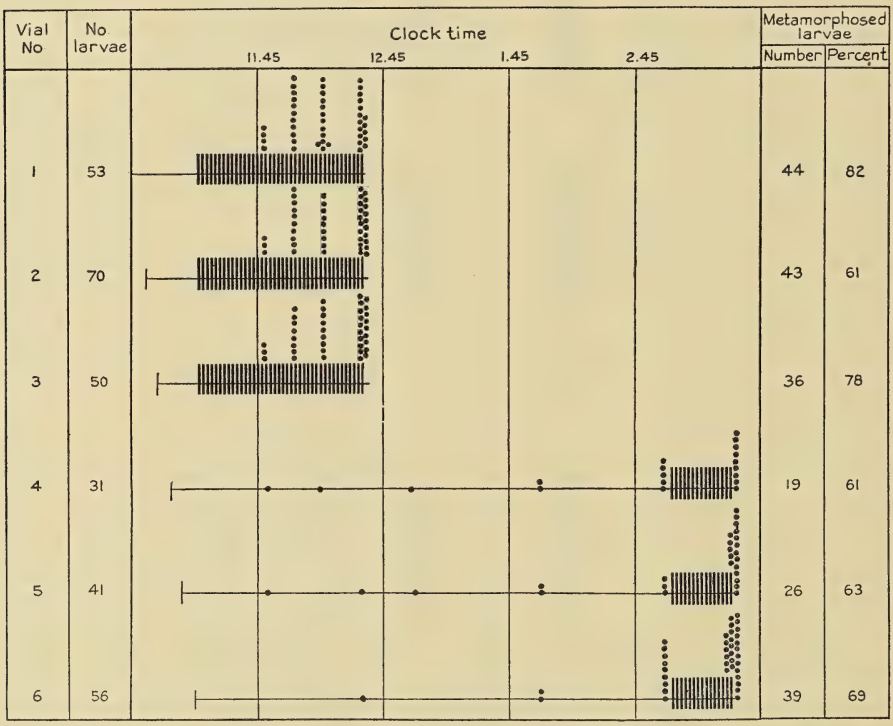
The possibility that the extreme variability in the time of development of this factor in the brooded larvæ of *Symplegma* and *Polyandrocarpa* may be due to differences in age of larvæ at the time of liberation was at first entertained, since it is not unlikely that each brood of larvæ represents a gradually increasing assemblage derived from eggs fertilized at different times and that some broods may be retained in the brood chambers longer than others. But when the same variability was found to characterize larvæ of *Phallusia nigra* about which no question of dissimilarity of age of individuals of a lot can arise, since all are derived from eggs artificially fertilized simultaneously, this explanation was seen to be invalid. Variability in the differentiation of susceptibility is probably correlated to some extent at least with differences in genetic constitution.



GRAPH 6: EXPERIMENT 81, August 17, 1929. Larvæ (*P. gravei*) liberated between 11:05 and 11:17, collected in 3 vials (5 x 1.8 cm.). Vials 1 and 2 placed on laboratory table, the former given three series of experimental treatment, as indicated on the graph, each consisting of 30 1-minute exposures to darkness and 30 alternating 1-minute exposures to the diffuse light of the laboratory. Vial 3 placed on an electric vibrator shelf and given three series of experimental treatments each consisting of 30 1-minute intervals of mechanical jarring and 30 alternating 1-minute intervals of quiet. Following the first treatment, examinations of vials 1 and 3 were made at intervals of 15 minutes between treatments and of vial 1 during each exposure to light. Vial 2, the control, was examined at 5:00 and 6:00 p.m. Black circles above horizontal line indicate the cases of metamorphosis that occurred. The condition of "resistance" to induction of metamorphosis is illustrated by this experiment.

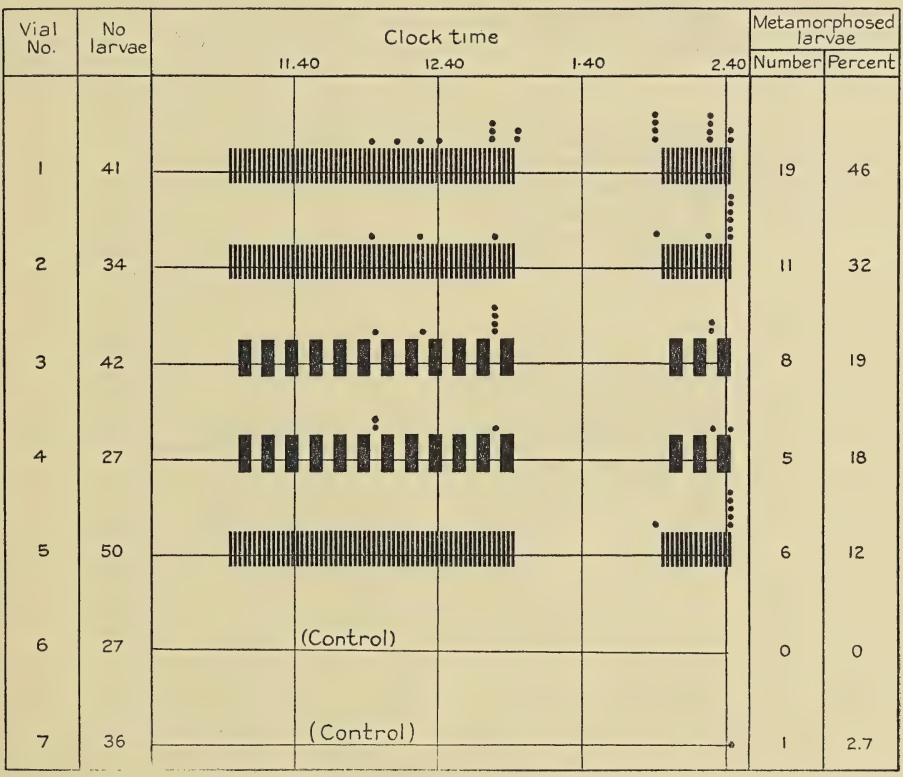
Whether or not development of susceptibility is wholly independent of environmental influence is not yet certainly known. That it is not affected by the various kinds of physical treatment that have been success-

fully used for the acceleration of metamorphosis is fairly well established, but the results of experiments carried on in 1933 in which larvæ were subjected to the influence of various chemicals seem to indicate that the differentiation of susceptibility may be subject to experimental acceleration. The fact that by concentrating a relatively large number of larvæ in a small volume of water metamorphosis is greatly accelerated may be explained as an effect of some soluble substance, emanating from the larvæ, upon the production of the susceptibility factor. These experiments are elsewhere more fully discussed (page 255). The very great acceleration of metamorphosis that has invariably occurred in larvæ subjected to the action of precipitates in sea-water produced by the addition of NaOH and KOH, discussed on page 261, and the early acceleration induced by various tissue extracts, may be due to an effect on the susceptibility mechanism. In all of these cases there seems to be no waiting for the differentiation of a susceptibility factor.

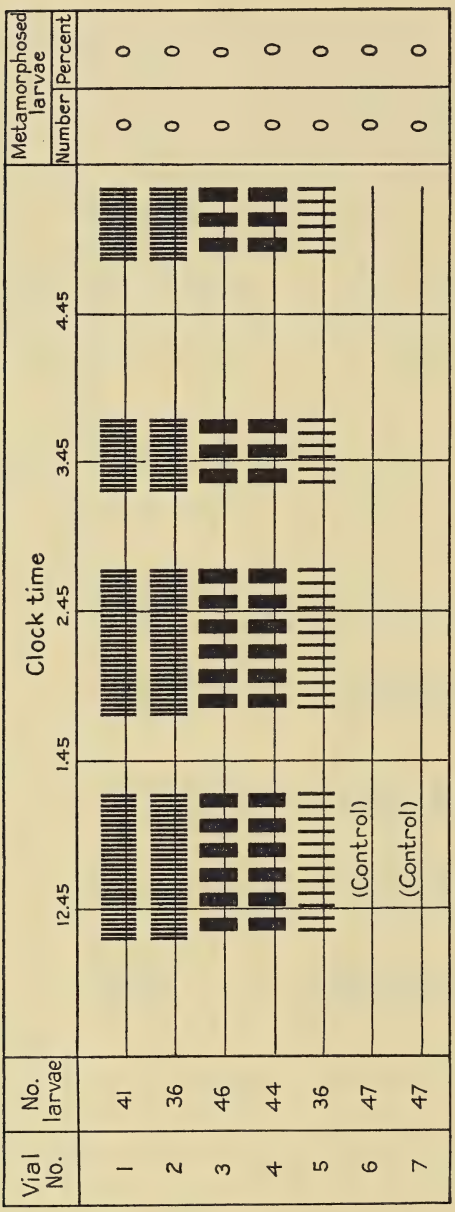


GRAPH 7: EXPERIMENT 111, July 24, 1930. Larvæ (*P. gravei*) liberated between 10:45 and 11:15 a.m., collected in 6 vials (5 x 1.8 cm.) which were placed, on the Mills Water Table. Vials 1, 2 and 3 were given 40 1-minute exposures to darkness each followed by a 1-minute exposure to diffuse light. At the conclusion of this treatment formalin was added to these vials. The larvæ in vials 4, 5 and 6 were untreated and served as the control until 3 p.m. when, four hours after their liberation, they were given a treatment consisting of 15 1-minute exposures to darkness each followed by a 1-minute exposure to the diffuse light of the laboratory. Formalin was then added and counts were made of the number of larvæ and metamorphosed zooids. Each black circle on the graph indicates the time of metamorphosis of a larva.

When the susceptibility factor appears, it persists for a time, but not indefinitely. Evidence has accumulated that it slowly wanes and finally disappears. While it is present, any method of experimental treatment that stimulates larvæ to swim will induce metamorphosis, but when it disappears no treatment, or resultant activity, will cause the transformation. The ability to metamorphose disappears with the loss of susceptibility. Control groups of larvæ that have stood undisturbed for several hours have consistently shown a small percentage only of metamorphosis when given the same treatment that induced metamorphosis of the entire treated group during the first hour of their free-swimming period, and it has been repeatedly observed that larvæ that long remain inactive usually die without having metamorphosed.



GRAPH 8: EXPERIMENT 118, July 29, 1930. Larvæ (*P. gravei*) liberated between 10:40 and 11:05 a.m., collected in a beaker from which 7 vials (5 x 1.8 cm.) were stocked and placed on the Mills Water Table. Beginning at 11:11, larvæ in vials 1 and 2 were given treatment consisting of 60 1-minute exposures to darkness, each followed by a 1-minute exposure to diffuse light; vials 3 and 4, 12 5-minute exposures to darkness each followed by a 5-minute exposure to diffuse light; vial 5, 60 1-minute intervals of mechanical jarring, each followed by a like interval without jarring. All experimental treatments were discontinued at 1:11 p.m. for an hour, then resumed for a period of 30 minutes, at the end of which formalin was added to all vials. Larvæ in vials 6 and 7 received no treatment and served as the control. Each black circle shown on the graph indicates the time of metamorphosis of a larva.



GRAPH 9: EXPERIMENT 121, July 31, 1930. Larvæ (*P. gravei*) liberated between 11:45 and 12:30 p.m. collected in a beaker and then distributed to 7 vials (5 x 1.8 cm.), all placed on the water table and treated simultaneously: vials 1 and 2 receiving four series of treatment, as indicated on the graph, consisting respectively of 30, 30, 15 and 15 1-minute exposures to darkness, each followed by a 1-minute exposure to diffuse light of the laboratory; vials 3 and 4 receiving four series of treatment consisting respectively of 6, 6, 3 and 3 5-minute exposures to darkness, each followed by a 5-minute exposure to diffuse light; vial 5 given four series of treatment consisting of respectively 12, 12, 6 and 6 volleys of air bubbles from a pipette at the bottom of the vial made at the beginning of each 5-minute interval, each volley carrying the larvæ from the bottom of the vial. Vials 6 and 7 stood undisturbed on the water table throughout the experiment. Examinations were made of all vials at frequent intervals following the first treatment, but no metamorphosis occurred. This experiment showed conclusively that larval activity alone, however stimulated, can not induce metamorphosis.

This characteristic of the susceptibility factor, as inferred from the observations cited and many others of like nature, is graphically represented in the text-figure (A). The free-swimming period of the *Polyandrocarpa* larva is represented by the continuous horizontal straight line, a-m; the susceptibility factor by the curves A, B and C, as inferred from results of experiments 53 (Graph 3), 81 (Graph 6) and 121 (Graph 9). Experimental treatment, to be effective in inducing metamorphosis in a larva with susceptibility as shown by the curve "A", must be given during the interval

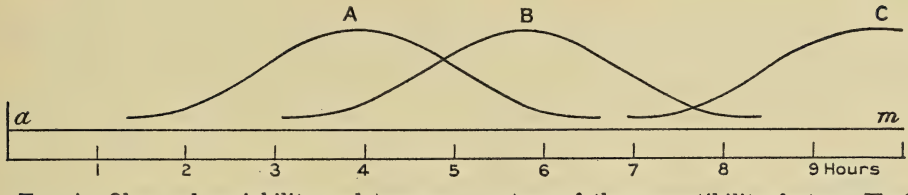
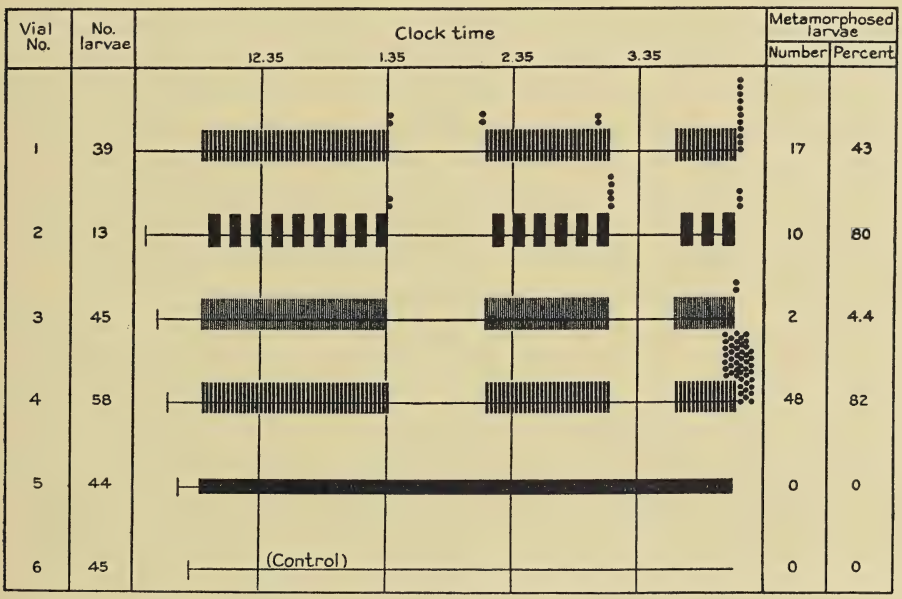


FIG. A—Observed variability and temporary nature of the susceptibility factor. Horizontal line, a-m, represents duration of free-swimming period of *Polyandrocarpa* larva below which time in hours is indicated. Curve A, susceptibility as it appeared, waxed and waned in Experiment 53. Curves B and C, susceptibility as interpreted from Experiments 81, 121 and others.

covered by the curve and most effectively at the time when the curve is at its mode. Experimental treatment given during the 6th hour occurs too late to have a maximum effect, some of the larvæ having lost their susceptibility. For larvæ with susceptibility as indicated by the curve "C", any treatment that occurs prior to the 7th hour can have no effect in inducing metamorphosis because no susceptibility factor has yet appeared.



GRAPH 10: EXPERIMENT 123, August 1, 1930. Larvæ (*P. tincta*) liberated between 11:35 and 12:02 were collected in a beaker from which 6 vials (5 x 1.8 cm.) were stocked and placed on the Mills Water Table. Beginning at 12:05 and ending at 4:20 p.m., experimental treatments were given simultaneously to the larvæ in vials 1, 2, 3 and 4, with an interval of rest between 1:35 and 2:20 and another between 3:20 and 3:50 p.m., as follows: Vial 1 received 90 1-minute exposures to darkness, each followed by a 1-minute exposure to diffuse light; vial 2 received 18 5-minute exposures to darkness, each followed by a 5-minute exposure to diffuse light; vial 3 received 180 mechanical agitations of the water once per minute; vial 4 received 90 1-minute mechanical jarrings each followed by a one-minute interval without jarring; larvæ in vial 5 were exposed to darkness throughout the experiment, while those in vial 6, constituting the control, were exposed to uniform conditions of diffuse light. Each black circle shown on the graph indicates the time of metamorphosis of a larva.

Throughout the summer of 1929 and part of 1930, the tentative assumption that the susceptibility factor is to be identified with or constitutes an important part of the larval mechanism of metamorphosis was held as a working hypothesis. It seemed likely that other factors were also involved, but none could be clearly visualized in the light of the many conflicting experimental results.

The hypothesis that metamorphosis may in some manner be causally related to larval activity was suggested in 1927 shortly after the beginning of the investigation but it then received scant consideration. Observations with which the investigation originated created too strong a bias in favor of light as the specific agent required to induce metamorphosis (see introductory section, page 212).

When in 1929 it was found that metamorphosis is also induced in larvæ by subjecting them to a series of mechanical jarrings, it was no longer possible to hold that light bears any specific relation to metamorphosis or that its mechanism is specifically organized for activation by light. Continued experiments in 1930 with mechanical treatment resulted in observations showing conclusively that it is successful in inducing metamorphosis only when, and to the extent that, it induces larvæ to swim. Larvæ of different broods were found to differ to a marked degree in their response to mechanical treatment. All of some broods began to swim at the instant they were jarred or agitated, but in others some were activated, others not. It was noted too that a variable percentage of larvæ of some broods, that were at first thrown into activity by the jarring or agitation treatment, sooner or later failed to respond, indicating that they become accustomed to this kind of stimulus. In experiments in which this failure of mechanical treatment to induce consistent activity was noted, a correspondingly low percentage of metamorphosis occurred, suggesting that the percentage of metamorphosis is correlated with the amount of activity of the larvæ. This definite correlation further suggested that, in the cases of methods of treatment with changes in light intensity also, the swimming activity induced may be the factor responsible for the metamorphosis that occurs. Observation of larvæ during treatment by the several methods devised supported this suggestion. A light-intensity change never fails to activate a larva, hence the consistently greater effectiveness of this method than that of other methods.

The implication of these observations could not be avoided. The effective part of all methods of treatment in the induction of metamorphosis is in some way due to neuro-muscular activity and not to any effect of light energy as such, or to any disturbance of internal equilibrium due to shocks transmitted to larvæ during treatment.

Despite the many observations that seemed at first impossible to harmonize with this theory, it became clear after scrutiny of certain experiment results, especially those with "resistant" lots of larvæ, that the *total* amount of larva activity occurring during a period of treatment is not necessarily

involved in the induction of metamorphosis, but only that part taking place *after* the "susceptibility" factor has been differentiated.

Earlier recognition of the essential rôle played by larval activity in the induction of metamorphosis was prevented by the numerous observations not in harmony with what was assumed to be the implication of the activity hypothesis; namely, that the percentage of metamorphosis occurring in a given experiment should be directly proportional to the amount of activity displayed by the larvæ. It was evident from the results of numerous experiments that no such correlation exists: the amount of activity occurring in the groups of "resistant" larvæ during the relatively long intervals of their ineffective treatment was many times greater than that of groups of "susceptible" larvæ that were induced to metamorphose after treatment with relatively few one-minute changes in light intensity. The number of metamorphoses that occurred in a group of larvæ stimulated to activity at intervals of 5 minutes was too nearly equal that occurring in groups of the same brood stimulated at one-minute intervals. The latter with five times the activity showed less than twice the percentage of metamorphosis (Graphs 8 and 10). The number of metamorphoses occurring in control lots of larvæ may also be cited: Vials containing these untreated control groups, that were handled at intervals of 30 minutes or an hour, often showed a percentage of metamorphosis entirely out of proportion to the amount of swimming this infrequent handling would excite, as compared with that of larvæ of the same brood that had been subjected to frequent stimulation (Graph 2). The fact that in earlier experiments light intensity changes made at intervals of one-minute were much more effective in the percentages of metamorphosis induced than the same made at intervals of 20 or 30 seconds finds its explanation in the activity hypothesis. The former are more effective in causing larvæ to swim. Larvæ respond to each stimulus at the longer intervals but not to each at the shorter, apparently because of failure of the larval neuromusculature to recover from fatigue in periods shorter than one minute. The almost complete failure of experiments in which treatment of larvæ consisted in interruptions of a beam of sun-light at intervals of one second, although continued for four hours, may be thus accounted for also.

The amount of activity necessary to induce metamorphosis after the susceptibility substance has appeared is much less than was suggested by the results of earlier experiments; these having indicated that the minimum amount of treatment required to cause metamorphosis consists of 25 or 30 one-minute exposures to light, each followed by a like interval of darkness, a treatment extending over an hour. The actual minimum of activity required to start the disruptive phase of metamorphosis may be estimated from results of experiments in which stimulation occurred at 5-minute intervals (see Graphs 8 and 10), and by the amount of metamorphosis that occurred in the control groups of experiments like that shown in Graph 2.

These data indicate that the activity resulting from a very few stimulations, if it occurs at the time the larva is at the height of its period of susceptibility, is sufficient to cause metamorphosis.

Thus obstacles to the acceptance of the idea that the induction of metamorphosis involves larval activity were removed and the way was paved for the formulation of a complete hypothesis of the mechanism of metamorphosis. The susceptibility factor may be assumed to be a chemical substance derived from some larval tissue or organ that is variable in the time of its differentiation. This substance is capable of combining with some by-product of neuro-muscular activity to form a third substance which, as it is produced and gradually assumes a higher and higher concentration in the tissue of the larval nervous system, has the property of stimulating the nervous system and thereby produces the series of events that constitutes metamorphosis. Its first effect is that of changing the adaptive responses of the larva to light and gravity; second, it causes the larva to attach to the side or bottom of the vial (in nature, to the under side of objects at the sea-bottom) and finally, in its highest concentration, it induces violent, tetanus-like muscle contractions by which the larval action system is completely disrupted and withdrawn into the body (page 224).

All observations and results of experiments seem to be in harmony with this hypothesis concerning the mechanism of metamorphosis. In larvæ of certain species of ascidian belonging to genera like *Perophora* and *Ecteinascidia,* in which the action system of the ascidiozooid is much better developed and not so completely inhibited as in the larvæ used in this investigation, the heart and blood vascular system frequently become functional in individuals that long remain inactive; and in such larvæ in which the blood has begun to circulate the disruptive phase of metamorphosis may not occur. The tail and papillæ may persist as appendages of the developing ascidian. In these unusual and peculiar cases, the failure to metamorphose may be accounted for on the assumption that neither the susceptibility substance nor the by-products of neuromuscular metabolism can reach a concentration necessary for metamorphosis, both being removed from the tissues by the circulating blood as rapidly as they form.

Species of ascidian are known in which metamorphosis of the larva may occur precociously within the brood chamber of the parent, the free-swimming period being eliminated. Such, according to observations made by Berrill (1931), is the case with the European form of *Molgula citrina.* In other species of the same genus, the larva is partially or entirely suppressed. Cases like the former may be brought into harmony with the proposed theory of metamorphosis by assuming that the susceptibility substance is precociously formed during the late embryonic development and that the amount of neuromuscular metabolism required for metamorphosis is provided by the series of periodic contractions that normally occur in the tail during the final stage of the embryonic tadpole within the chorionic mem-

brane. Such contractions are frequent and visible, their function being that of facilitating the hatching process by causing the rupture of the chorion. In the cases of degenerate tadpoles in species having so-called direct development, the larval action system is only partially developed and rudimentary, and it may be assumed that nothing comparable with metamorphosis takes place.

Metamorphosis occurs spontaneously under laboratory conditions in a larger or smaller percentage of larvæ apparently in the absence of any great amount of swimming activity, but much later than if frequently stimulated to swimming activity by artificial means, especially during the latter part of the free-swimming period when they tend to lie motionless for relatively long intervals. When metamorphosis occurs in larvæ that have not been made repeatedly active by artificial means, it usually lacks certain of its normal adaptive aspects. It is not usually preceded by attachment; the disruptive process is slower in running its course; is sometimes incomplete and the ascidiozooids float at the water surface. It is as if the feeble swimming movements of short duration that occasionally occur spontaneously in these quiescent larvæ produce an insufficient amount of the necessary by-product of neuromuscular activity for the requirements of the reaction that precedes and activates normal metamorphosis.

In their normal habitat larvæ doubtless receive a more or less continuous series of stimuli not present under laboratory conditions. As they are carried about by ever present currents, they doubtless frequently pass beneath the opaque bodies of animals and floating objects, and the alternate shadows and illuminations that are thus produced are effective activators or causes of swimming movement. During the later part of the free-swimming period larvæ are carried by their changing orienting responses to light and gravity from one level of light intensity to another in their downward and upward excursions between the bottom and water surface. A third natural condition that would lead to repeated stimulation to activity is that connected with the continual turning of the body of a larva, as it is transported by the tide, by which it is frequently brought into the position at which light rays enter the optic cup and stimulate the light-sensitive elements of the sense organ.

A theory of the cause of metamorphosis in ascidian larvæ, similar in one of its factors to that to which this study has led, has been advanced by Berrill (1929). He concludes from work done chiefly on the larva of *Phallusia mammillata,* that larval activity makes an important contribution to conditions required for metamorphosis by causing an increase in the intercellular acidity of the tissues of the tail, possibly by the accumulation of lactic acid, thus creating the hydrogen-ion concentration necessary for phagocytosis. The primary rôle of releasing the disruptive phase of metamorphosis he seems to assign to phagocytosis. The following are quotations from Berrill's paper.

Page 60, "The whole process [metamorphosis] can be controlled through the hydrogen-ion concentration of the medium." . . . Any increase in hydrogen-ion concentration tends to induce metamorphosis, while low concentrations, *e. g.*, pH 9.0, inhibit metamorphosis to a greater or less extent."

Page 61, "It seems possible that the onset of phagocytosis of the tail is caused primarily by an increasing acidity of the tissues concerned, and that normally this is brought about by the activity of the tadpole. Intercellular acidity in the tail could be the result of lactic acid accumulation, or of carbon dioxide, though the latter probably would diffuse away too quickly to have much effect."

"The transference of tadpoles from water of a high pH to water of a low pH produces a change in the intercellular fluid analogous to that produced as a result of muscular activity of the tadpole, and it is this change which induces phagocytosis and probably metamorphosis generally."

Page 64, "So here again (in *Styelopsis grossularia*) it seems that it is the sudden activity, and therefore an increase in intercellular acidity, that is effective" [in causing metamorphosis].

Page 65, "These results support the suggestion that the primary factor inducing phagocytosis in the ascidian tadpole is an increase in intercellular acidity."

In the general conclusion that larval activity plays an important rôle in the induction of metamorphosis, we are agreed, but Berrill's findings and interpretation of the contribution made by this activity in conditioning larvæ for metamorphosis are at almost complete variance with the observations and conclusions reported in this paper. The pH of the medium, and presumably therefore of the larval tissues, plays no significant part in the induction of metamorphosis. Metamorphoses occurring in experiments in which the pH of the sea-water medium was lowered or raised by the addition of substances such as lactic acid, sodium or potassium hydroxide, were due not to any effect of the pH, but to some effect of the substances causing the pH change. These experiments are described and discussed in a part of this report beginning on page 261. Berrill's suggestion that lactic acid could be the specific by-product of larval activity by which metamorphosis is induced was verified in experiments described on page 270, but no evidence was found that lactic acid acts in the way postulated by him—by producing a condition of intercellular acidity and thus conditioning phagocytosis. Neither was there evidence that phagocytosis is involved in the initation of metamorphosis. After metamorphosis has occurred, when the notocord, muscle bands and other tail tissues have been withdrawn into the body of the ascidiozooid, phagocytosis may be involved in their gradual disintegration and preparation for absorption.

As may be seen in the part of the text dealing with the results of experiments carried out in 1933, numerous substances were found to induce metamorphosis quickly and effectively, often to the extent of 100 per cent, the controls showing none. In all of these cases the pH of the medium was measured both at the beginning and at the end of an experiment. In

some it was low, in others high, but in the greater number the pH was that of normal sea-water at the Tortugas.

ACCELERATION OF METAMORPHOSIS BY CHEMICAL SUBSTANCES

To throw further light on the processes involved in metamorphosis was the aim of work planned for the summer of 1933. It was proposed to subject groups of larvæ to the effect of a number of substances known to be products of metabolism, the possibility being foreseen that in this way the identity of one or more of the substances assumed to constitute the mechanism of metamorphosis might be revealed.

The substances provided were lactic acid (Baker and Adamson), pituitrin whole gland extract, pituitrin "O," anterior lobe extract, thyroid whole gland extract (Parke Davis and Company) and acetylcholine (Eastman Kodak Company). Cylinders of oxygen and carbon dioxide and various chemicals for changing the pH of sea-water media were available. Results of experiments with thyroid whole gland extract led to the preparation and use of various fresh animal-tissue extracts.

The use of electrical equipment for the accurate determination of hydrogen-ion concentrations of experimental media was attempted unsuccessfully and a colorometric method was substituted involving the use of LaMotte standard indicators and a Hellige block comparator, No. 602.

Larvæ of *Phallusia nigra* were available for work every afternoon in very large numbers, and broods of larvæ were liberated during the forenoon, several days each week, by large colonies of *Polyandrocarpa tincta.*

A large number of experiments were made which are described in the approximate order carried out, for it frequently happened that the results of one set of experiments suggested work for the following day.

DIFFERENTIATION- AND SWIMMING-WATER

It was incidentally noted in 1929 that metamorphosis in the *Phallusia* larva occurs much sooner and in higher percentages in beakers and vials that contain a relatively large number of larvæ than in containers of the same size stocked with few. One of the experiments made in 1930 in which this crowding effect is strikingly apparent especially in the control vials, 7 and 10, is summarized in table 8. The effect may be caused by some soluble product of larval metabolism which, by gradually increasing concentration in the water, finally reaches a point at which it has the property of initiating the process of metamorphosis. That it is not to be attributed to a diminution of the oxygen content of the water or to a high concentration of carbon dioxide is proved by the results of experiments with these gases, elsewhere described (page 257).

A systematic attempt was made in 1933 to solve this problem. By filtering the water from beakers and vials in which concentrated lots of larvæ

TABLE 8—*Experiment No. 90: 7, 2½'30. Phallusia nigra, Eggs fertilized 6:15 a. m. Hatching 2:15 p. m. Six vials stocked at 2:45-2:50 p. m. Four vials stocked at 3:20-3:25 p. m. Effect of large numbers of larvæ clearly shown.*

Vial number	Size of vial	Volume of water	Age of larvæ	Number of larvæ	Kind of treatment	Duration of treatment	Metamorphosed larvæ	
							Number	Per cent
1	5 × 2.2 cm.	13 cc.	30 min.	100	Control (untreated).	1 hr. (2:50–3:50).	0	0
2	"	"	"	101	30 1-min. exposures to diffuse light, each followed by 1-min. exposure to darkness.	"	2	2
3	"	"	"	76	60 momentary agitations of the water at beginning of 1-min. intervals.	"	1	1.3
4	"	"	"	32	Control.	2 hrs. (2:50–4:50).	0	0
5	"	"	"	140	60 1-min. exposures to diffuse light, each followed by 1-min. exposure to darkness.	"	12	8.5
6	"	"	"	74	120 momentary agitations of the water made at 1-min. intervals.	"	5	6.7
7	"	"	1 hr.	483	Control.	2 hrs. (3:25–5:25).	163	33.7
8	"	"	"	322	45 1-min. exposures to diffuse light, each followed by a 1-min. exposure to darkness.	1½ hrs. (3:25–4:55).	77	23.9
9	"	"	"	284	90 momentary agitations of the water made at 1-min. intervals.	"	12	4.2
10	8 × 2.2 cm.	25 cc.	"	1190	Control	2 hrs. (3:25–5:25).	590	49.5

had been swimming actively for two hours or more, supplies of differentiation or swimming water were repeatedly collected and used in 27 carefully controlled experiments, but the results are so conflicting and inconsistent that no detailed report can be made at this time. In 9 of these experiments the percentage of metamorphosis that occurred in groups of larvæ in such media was greatly in excess of that in control larvæ in pure sea-water, but in 18 experiments of exactly the same kind, either no acceleration of metamorphosis took place in the treated larvæ or it was not sufficiently greater than in the controls to be considered significant.

Considering the fact that crowding of larvæ has invariably resulted in a very great acceleration of metamorphosis, it is difficult to account for the failure of so large a percentage of these experiments to show a similar acceleration, but two suggestions present themselves: the by-product of larval metabolism assumed to be responsible for the acceleration that occurs when thousands of larvæ are crowded together in a small quantity of water may be an unstable compound that quickly disintegrates or combines with other substances in sea-water; or the acceleration that takes place under these conditions may be due, not to the action of a chemical substance, but to some physical effect, or simply to the numerous contacts of larva with larva which stimulate continuous swimming activity and hence induce metamorphosis at the earliest moment after the appearance of the susceptibility factor.

OXYGEN AND CARBON-DIOXIDE

The acceleration of metamorphosis invariably occurring when relatively large numbers of larvæ are crowded in small volumes of water raised the obvious question whether it may not be change in the oxygen or carbon-dioxide content of sea-water, resulting from metabolism of swimming larvæ that determines the duration of the free-swimming period. Experiments were therefore planned to ascertain the effect on groups of larvæ of reducing the oxygen and carbon dioxide content of sea-water below that normally present, and of increasing the sea-water content of both to saturation.

These sea-water media were prepared as follows: Oxygen was removed by bringing flasks of sea-water to the boiling point in completely filled flasks which were then tightly stoppered during the period of cooling and until experimental vials were filled. During an experiment cover-glasses were placed on the tops of the vials to prevent absorption of oxygen from the air.

The device by which sea-water was saturated with oxygen before and during an experiment consisted of a flexible glass tube of capillary diameter about 4 feet in length by which an oxygen reservoir was connected with the column of water in each experimental vial, the free end of the capillary tube entering the vial vertically and extending to a point near the bottom. Sea-water so treated had a pH value of 8.4. The continuous rising

stream of pure oxygen bubbles had no apparent disturbing or stimulating effect on the larvæ.

An exactly similar method was employed to saturate sea-water in experimental vials with carbon-dioxide, expired air under pressure being conveyed from a reservoir to the vials by relatively long capillary glass tubes. The pH value of sea-water thus saturated with the carbon-dioxide of expired air was about 7.2.

CO_2-free sea-water was prepared by drawing air through a series of flasks partially filled with media as follows: Flask A contained sea-water in CO_2 equilibrium with the air; KOH solution in flask B; distilled water slightly acidulated with HCl in flask C; pure distilled water in flask D. The sea-water in flask E became CO_2 free as the process continued several (16 to 20) hours. The tops of vials stocked with this CO_2-free sea-water (plus larvæ) were covered during experiment with cover-glasses, except for the intervals when the upper stratum of the medium was removed with a pipette and replaced from the stock of CO_2-free sea-water after the larvæ had largely settled. The pH value of the CO_2-free medium in the vials was about 8.9 or 9.

From the results of a limited number of experiments with these media, shown in tables 9 and 10, it may be noted that oxygen-free and carbon-dioxide-saturated sea-water both tended to inhibit metamorphosis. This is the effect logically to be expected, assuming that oxidative reactions are involved in the process of metamorphosis, but it is not in harmony with the observation that metamorphosis is consistently accelerated to a very marked degree when numerous larvæ are crowded in a small volume of water, for under these conditions the oxygen supply of the water would soon become depleted and the carbon-dioxide content increased by the metabolism of the swarm of swimming larvæ.

In the two experiments in which the effect of oxygen-saturated sea-water was tested, the results are somewhat contradictory, a slight acceleration of metamorphosis having occurred in one, a slight inhibition in the other. The effect of an increase in the amount of available oxygen is probably negligible.

Carbon-dioxide-free sea-water, however, had a marked accelerating effect in each of the four experiments in which it was used: three with *Phallusia* larvæ, one with *Polyandrocarpa*. How this effect is to be explained is no clearer than the unexpected inhibiting effect of sea-water saturated with carbon-dioxide and of oxygen-free sea-water. Explanation must wait upon further knowledge of the chain of internal events leading up to and constituting the process of metamorphosis.

Dr. C. M. Child (1927), from a study of conditions under which development and differentiation occur in the ascidian *Corella willmeriana*, came to the conclusion that in this species a higher CO_2 content than is found in normal sea-water has become an important and controlling factor in devel-

TABLE 9—*Experiment No. 145: 7, 12, '33. Oxygen lack. Phallusia larvæ.*

Number of vial	Size of vial	Capacity of vial	Age of larvæ	Number of larvæ	Medium	pH.	Duration of experiment	Metamorphosed larvæ	
								Number	Per cent
1	6 × 1.8 cm.	9 cc.	30 min.	136	Sea-water with O removed by boiling.*	8.4	6 hrs. (2–8 p.m).	15	11
2	"	"	"	128	" " " " " " "	"	"	24	18
3	"	"	"	150	" " " " " " "	"	"	35	23
4	"	"	"	127	" " " " " " "	"	"	27	21
5	"	"	"	120	" " " " " " "	"	"	24	20
6	"	"	"	131	Normal sea-water. Control.	"	"	61	46.5
7	"	"	"	123	" " " "	"	"	57	46.3
8	"	"	"	113	" " " "	"	"	35	31
9	"	"	"	117	" " " "	"	"	29	24.7
					Experiment No. 146: 7, 12, '33. Oxygen lack. Phallusia larvæ.				
1	8 × 2.2 cm.	25 cc.	40 min.	322	Sea-water with O removed by boiling.*	8.4	5⅝ hrs. (2:10–8 p.m.).	67	20.8
2	"	"	"	317	" " " " " " "	"	"	35	11
3	"	"	"	342	Normal sea-water. Control.	"	"	169	49.4
4	"	"	"	335	" " " "	"	"	168	50

* Vials containing "O" free sea-water covered with cover-glasses during experiment.

TABLE 10—*Experiment No. 153: 7, 18, '33. CO_2 saturated sea-water. Phallusia arvæ.*

Number of vial	Size of vial	Capacity of vial	Age of larvæ	Number of larvæ	Medium	pH.	Duration of experiment	Metamorphosed larvæ	
								Number	Per cent
1	6 × 1.8 cm.	9 cc.	30 min.	142	Normal sea-water. Control.	8.4	6 hrs. (2:10–8:10 p.m.).	5	3.5
2	"	"	"	148	" " " "	8.4	"	4	2.7
3	"	"	"	140	" " " "	8.4	"	2	1.4
10	"	"	"	116	CO_2 saturated sea-water.	7.2	"	0	0
11	"	"	"	141	" " " "	7.2	"	0	0
12	"	"	"	128	" " " "	7.2	"	4	3
Experiment No. 162: 7, 23, '33. CO_2 saturated, CO_2 free and O saturated sea-water. Phallusia larvæ.									
1	8 × 2.5 cm.	27 cc.	25 min.	813	CO_2 saturated sea-water (bubbling respired air).	7–7.6	5 hrs. (1:35–6:35).	87	11.9
2	"	"	"	877	CO_2 free sea-water.	9–8.9	"	491	55.9
3	"	"	"	769	Oxygen saturated sea-water (bubbling pure O).	8.4	"	190	24.7
4	"	"	"	781	Natural sea-water. Control.	8.4	"	240	30.7
Experiment No. 164: 7, 24, '33. CO_2 saturated, CO_2 free and O saturated sea-water. Phallusia larvæ.									
1	8 × 2.5 cm.	27 cc.	30 min.	623	CO_2 saturated sea-water (bubbling respired air).	(?)	8 hrs. (1:45–9:45 p.m.).	14	2.2
2	"	"	"	650	CO_2 free sea-water. (Vial covered during exp't.)	(?)	"	590	90
3	"	"	"	472	Oxygen saturated sea-water (bubbling pure O).	(?)	"	30	6.3
4	"	"	"	546	Normal sea-water. Control.	(?)	"	16	2.9
Experiment No. 165: 7, 24, '33. CO_2 free sea-water. Polyandrocarpa larvæ.									
1	8 × 2.5 cm.	27 cc.	5–15 min.	33	Normal sea-water. Control.	8.4	4⅛ hrs (9:40–2 p.m.).	1	3
6	"	"	"	73	" " " "	8.4	4⅙ hrs. (9:50–2 p.m.).	2	2.7
7	"	"	"	49	CO_2 free sea-water. (Vial covered during exp't.)	9	4 hrs., 5 min. (9:55–2 p.m.).	44	89
Experiment No. 168: 7, 26, '33. CO_2 free sea-water. Phallusia larvæ.									
1	6 × 2 cm.	13 cc.	30 min.	118	Normal sea-water. Control.	8.4	4 hrs. (2:25–6:30 p.m.).	3	2.5
2	"	"	"	93	" " " "	8.4	"	0	0
3	"	"	"	139	CO_2 free sea-water	9	"	43	30
4	"	"	"	117	" " " "	9	"	34	29

opment. In this species the eggs and embryos are incubated in the atrial chamber of the parent within which the water was found to have a pH ranging from 7.0 to 7.6 "ordinarily due to CO_2 resulting from respiration and readily removed by shaking with air, but the fæces contain some acid, to which the increase in the CO_2 content is due. When developing eggs were transferred from the atrial chamber to normal sea-water, differential inhibition of development occurred"—the inhibited forms showing all degrees of reduction and elimination of the larval stage and approaching direct development and sometimes hatching in an advanced stage of metamorphosis—"under the more extreme inhibiting conditions metamorphosis may be retarded or even stopped." The inhibiting factor is not the altered pH, but the change in the CO_2 content of the water. "Normal development does not depend on the pH alone but nevertheless apparently does depend on the amount of acid added (from the fæces) to the sea-water. This being the case, two possibilities apparently exist; either the anion of the acid inhibits above a certain concentration or else change in the sea-water resulting from the addition of acid is the effective factor." Since the amount of acid added is very small in all cases, inhibiting action of the anion appears to be excluded. The chief change in the sea-water brought about by the addition of acid is an increase in the CO_2 content."

This adjustment of development in *C. willmeriana* to a higher CO_2 content than is found in normal sea-water is a secondary one not occurring in many species, and the differential inhibition of development that occurs when eggs of this species are transferred to water with a normal (lower) CO_2 concentration therefore affords no basis for an interpretation of the effect on metamorphosis of changes in the CO_2 content of sea-water described in this paper.

NaOH, KOH, $BaCl_2$, $AgNO_3$, NaCl, KCl and HCl

Experiments with sodium and potassium hydroxide and hydrochloric acid were planned with the aim only of testing the validity of the conclusion reached by Berrill (1929): He states, page 75—

"the onset and rapidity of metamorphosis of *Phallusia, Ascidiella* and *Ciona* tadpoles may be controlled by varying the hydrogen ion concentration of the water. Hyperalkaline sea-water tends to inhibit metamorphosis altogether, while changes in the direction of increased acidity tend to induce metamorphosis."

When it was found that a slight but definite inhibiting effect on metamorphosis followed the addition of HCl (experiment No. 159, table 15) and that increases in the alkalinity of sea-water due to the addition of either NaOH or KOH were effective in accelerating metamorphosis only

when the pH was raised above 9.4,[1] at which value the $Mg(OH_2)$ formed becomes insoluble and precipitates, it became apparent that the accompanying changes of pH in these sea-water media are factors of only secondary importance.

That the increase in positive cations, Na+ and K+, are also unimportant in inducing metamorphosis was demonstrated by increasing their concentration in the sea-water, to the same value as that reached when their hydroxides were added, by the addition of sodium or potassium chloride. Increasing either of these chlorides had, of course, no effect on the pH value and the changes in either Na^+, K^+, or Cl^- concentrations did not increase metamorphosis, as may be seen from experiment No. 187 b and c (table 14). Considering the already high concentrations of these ions in Tortugas sea-water, negative results were to be expected.

The accelerating action of the NaOH or KOH must therefore be in some way connected with the formation of the insoluble $Mg(OH)_2$, or with the physical presence of the precipitate in the sea-water media.

The possibility that the hydroxide precipitate produced its accelerating effect in a purely physical manner, either by offering an absorbing surface for some inhibitory substance present in the media, or as a mechanical stimulus to larval activity, seems to be excluded by two experiments in which other finely divided insoluble substances were added to the sea-water medium: excess soluble starch and animal charcoal, neither of which had any effect on the rate of metamorphosis.

Experiments to determine the nature of the accelerating effect of the hydroxide precipitates were continued and extended to include other precipitate-forming substances: barium chloride and silver nitrate. Also two experiments were performed in which potassium oxalate was added, yielding heavy precipitates of calcium oxalate, which showed that neither the lack of calcium nor the presence of this precipitate were effective in accelerating metamorphosis.

These several experimental sea-water media were prepared as follows: NaOH solutions were made up in four ways: (1) immediately before use a sufficient quantity of NaOH was added to sea-water to bring the final normality of *added NaOH to 0.0086*; (2) twelve hours before use, sufficient

[1] As stated elsewhere, pH determinations at Tortugas were made with indicators and colored glass standards. The values so obtained are somewhat inaccurate, due to the high salt concentration. Hence, during the summer of 1934, filtered sea-water was shipped from Miami to the laboratory in St. Louis which was used for a series of determinations of the pH changes on addition of acid or base, by means of the glass electrode. It was found that when the normalities of added NaOH or KOH were equal to those used at Tortugas, no precipitate appeared until an initial pH of approximately 9.4 was reached. This precipiate was analysed and found to consist chiefly of $Mg(OH)_2$. No $Ca(OH)_2$ was precipitated at these pH values (A. R. Haas, 1916, Jour. Biol. Chem., vol. 26, 315). After standing 24 hours, the pH dropped to points between 8.6 and 8.9, due apparently to absorption of CO_2. These values, though probably not identical with those occurring in experimental media at Tortugas, due to differences in CO_2 tension, lack of organisms in the water, etc., do corroborate the range of values worked with and the direction of change of pH values under experimental conditions.

NaOH was added to sea-water to bring the final normality, after all dilutions, to 0.0086 N. of added NaOH; (3) identical with (2) except that the precipitate which had formed was filtered off (Whatman's No. 40); (4) identical with (2) except that the precipitate was not filtered off, the supernatant fluid being transferred to the vials with a pipette.

Potassium hydroxide solutions corresponding to those of NaOH, just described, were prepared, the final normality of added KOH being either 0.0066 N. or 0.0044 N. The pH of these solutions were similar to those of the NaOH solutions.

In order that the time of appearance of the precipitate should be delayed in some of these experiments, the molar value of the added NaOH of KOH was varied in a series of concentrations just below that required to produce a precipitate immediately: Experiments 175, 183, 186 and 187 in tables 11 and 12.

The molar value of the added barium chloride was either 8.3×10^{-4} or 5×10^{-5} including the dilution that took place when larvæ were placed in the vials. Larvæ were added to the vials before the $BaCl_2$ solution was introduced. The pH remained that of normal sea-water.

The final molar value of added silver nitrate was either 1.1×10^{-4} or 2.0×10^{-5}. The $AgNO_3$ solutions were introduced into the vials after they were stocked with larvæ. No change in pH occurred.

In the case of HCl approximately 0.1 N. was added to sea-water and the solution then equilibrated to the partial pressure of the $C0_2$ of the air, by bubbling air through for twelve hours. The equilibrated pH was 7.4. Due to the addition of a small quantity of fresh sea-water when the larva were placed in the vials, the pH rose slightly during an experiment.

Five of the experiments made with NaOH media, two with KOH and one each with $BaCl_2$, $AgNO_3$, NaCl, KCl and HCl are summarized in tables 11 to 15.

The most rapid and consistent acceleration of metamorphosis that has occurred in any of the entire series of experiments described in this paper took place in NaOH and KOH media.[1] A critical consideration of the data of these and other experiments of similar nature leads to the conclusion that the effective agent or condition is not the pH changes of the media but some substance formed in the precipitate thrown down when NaOH, KOH or

[1] Metamorphosis in these media often differs from normal in that disruption of the tail does not occur immediately after attachment. Later, when the disruptive process takes place, it progresses slowly and often incompletely, a longer or shorter terminal part of the notocord protruding beyond the body of the zooid. Observations suggest that the larval tunic becomes adhesive in these media and that this may account for the very early attachment that occurs. In many cases, especially in larvæ of *Polyandrocarpa*, the entire tunic not only becomes adhesive but tends to dissolve, become loosened from the body and to be sloughed off. Such seemingly abnormal zooids, of both *Phallusia* and *Polyandrocarpa*, isolated frequently in fresh sea-water, have in all cases developed normally and rapidly into the adult form.

TABLE 11—*Experiment No. 155. 7, 19, '33. NaOH. Phallusia.*

Number of vial	Size of vial	Capacity of vial	Age of larvæ	Number of larvæ	Medium	pH.	Duration of experiment	Metamorphosed larvæ	
								Number	Per cent
1	6 × 1.8 cm.	9 cc.	30 min.	61	0.0086 N. NaOH (fresh). ppt. came immediately.	9.1–9.1	3⅚ hrs. (2:40–6:30 p.m.).	49	88
2	"	"	"	77	" " "	"	"	59	76
3	"	"	"	77	" " "	"	"	67	87
4	"	"	"	105	" (old). ppt. filtered out.	8.9–8.9	"	9	8
5	"	"	"	118	" " "	"	"	5	4
6	"	"	"	105	" " "	"	"	12	11
7	"	"	"	64	" ppt. not removed.	9–9	"	3	4.7
8	"	"	"	98	" " "	"	"	8	8
9	"	"	"	98	" " "	"	"	15	15
10	"	"	"	87	" (fresh). No ppt. formed.	9.1–9.1	"	4	5
11	"	"	"	84	" " "	"	"	4	5
12	"	"	"	99	" " "	"	"	29	29
13	"	"	"	108	" (old). ppt. filtered out.	8.8–8.6	"	0	0
14	"	"	"	116	" " "	"	"	4	3
15	"	"	"	94	" " "	"	"	6	5
16	"	"	"	106	Sea-water. Control.	8.4	"	5	4.9
17	"	"	"	88	" "	8.4	"	7	7.9
18	"	"	"	85	" "	8.4	"	7	8.2
Experiment No. 186. 8, 4, '33. NaOH. Phallusia.									
9	6 × 1.8 cm.	9 cc.	30 min.	176	0.0044 N. NaOH. Made up 12 hrs.—ppt. removed.	(?)	3½ hrs. (1:40–5:10 p.m.).	7	4
10	"	"	"	194	" " "	(?)	"	7	3.6
11	"	"	"	119	0.0066 N. " "	(?)	"	21	17
12	"	"	"	210	" " "	(?)	"	12	5.7
1	"	"	"	151	Sea-water. Control.	(?)	"	17	11.3
2	"	"	"	162	" "	(?)	"	7	4.3

TABLE 12—*Experiment No. 175. 7, 29, '33. NaOH. Polyandrocarpa.*

Number of vial	Size of vial	Capacity of vial	Age of larvæ	Number of larvæ	Medium		pH.	Duration of experiment	Metamorphosed larvæ	
									Number	Per cent
1	8 × 2.5 cm.	27 cc.	3-18 min.	58	Sea-water.	Control.	8.4	6⅚ hrs. (10:10–5:00 p.m.).	7	12
3	"	"	"	50	NaOH 0.0012 N.	ppt. visible at 4.30.	9.1	6⅛ hrs. (10:17– ").	2	4
4	"	"	"	76	" 0.0013 N.	" " " 4.00.	9.2	6⅛ hrs. (10:19– ").	3	3.9
6	"	"	"	72	Sea-water.	Control.	8.4	6⅛ hrs. (10:20– ").	3	4
Experiment No. 183. 8, 2, '33. NaOH. Polyandrocarpa.										
1	8 × 2.5 cm.	27 cc.	7–30 min.	46	Sea-water.	Control.	8.4	4 hrs., 23 min. (10:22–2:45 p.m).	0	0
2	"	"	"	47	NaOH 0.0014 N.	ppt. filtered off.	8.8	4⅛ hrs. (10:25–2:45 p.m.).	0	0
3	"	"	"	64	" 0.0021 N.	" " "	9.0	4 hrs., 18 min. (10:27–2:45 a.m.).	0	0
4	"	"	"	35	" 0.0014 N.	ppt. visible at 12.40.*	(?)	3½ hrs. (10:30–2:00 p.m.).	35*	100
5	"	"	"	53	" 0.0021 N.	" " " 11.03.	9.2	2 hrs. (10:35–12:30 p.m.).	53	100
8	"	"	"	43	" 0.0021 N.	" " " 10.48.	9.2	42 min. (10:48–11:30 a.m.)	43	100
7	"	"	"	56	Sea-water.	Control.	8.4	4 hrs. (10:45–2:45 a.m.).	0	0
Experiment No. 187. 8, 4, '33. NaOH. Polyandrocarpa.										
1	8 × 2.5 cm.	27 cc.	8–30 min.	31	Sea-water.	Control.	8.4	7 hrs., 39 min. (9:31–5:10 p.m.).	0	0
3	"	"	"	41	NaOH 0.0021 N.	ppt. not filtered off.**	8.8–8.7	1 hr., 43 min. (9:37–11:20 a.m.).	38	92
4	"	"	"	27	NaOH 0.0014 N.	" " " " **	9.2–8.9	1⅚ hrs. (9:40–11:30 a.m.).	23	85
9	"	"	"	50	Sea-water.	Control.	8.4	7 hrs., 18 min. (9:52–5:10 p.m.).	0	0

* None metamorphosed at 12:40 when ppt. appeared, 3 metamorphosed at 1:10 p.m.; 16 at 1:30; all at 2:00.
** ppt. on bottom of vial.

TABLE 13—*Experiment No. 186: 8, 4, '33. KOH. Phallusia,*

Number of vial	Size of vial	Capacity of vial	Age of larvæ	Number of larvæ	Medium		pH.	Duration of experiment	Metamorphosed larvæ	
									Number	Per cent
1	6 × 1.8 cm.	9 cc.	30 min.	151	Sea-water.	Control.	8.4	3½ hrs. (1:40–5:10 p.m.).	17	11.3
2	"	"	"	162	"	"	8.4	"	7	4.3
3	"	"	"	119	KOH 0.0022 N.	ppt. visible at 3 p.m.	(?)	"	49**	41
4	"	"	"	114	" "	" " " "	(?)	"	45**	40
5	"	"	"	133	" "	ppt. settling (made up 4 hrs.).*	(?)	"	28**	21
6	"	"	"	127	" "	"	(?)	"	37**	29

* ppt. settled on bottom; larvæ introduced carefully, not to disturb ppt.
** Zooids and larvæ buried in ppt. and difficult to count.

Experiment No. 187a: 8, 4, '33. KOH. Polyandrocarpa,

1	8 × 2.5 cc.	27 cc.	8–30 min.	31	Sea-water.	Control.	8.4	7 hrs., 39 min. (9:31–5:10 p.m.).	0	0
2	"	"	"	38	KOH 0.0022 N.	ppt. settled (made up 12 hrs.).	9–8.9	1¾ hrs. (9:35–11:20 a.m.).	38	100
5	"	"	"	32	KOH 0.0022 N. (fresh).	ppt. came at 10:50.	9.3	2 hrs. (9:43–11:45 a.m.).	23	85
9	"	"	"	50	Sea-water.	Control.	8.4	7 hrs., 18 min. 9:52–5:10 p.m.).	0	0

TABLE 14—*Experiment No. 184. 8, 3, '33. Barium chloride. Phallusia.*

Number of vial	Size of vial	Capacity of vial	Age of larvæ	Number of larvæ	Medium		pH.	Duration of experiment	Metamorphosed larvæ	
									Number	Per cent
1	6 × 1.8 cm.	9 cc.	30 min.	196	Sea-water.	Control.	8.4	6 hrs. (1:40–7:40 p.m.).	41	20
2	"	"	"	246	"	"	"	"	19	7.7
3	"	"	"	196	"	"	"	"	9	4.5
12	"	"	"	98	$BaCl_2$.	0.15 cc. of M/20 sol. (copious ppt.).	(?)	"	98	100
13	"	"	"	84	"	"	(?)	"	84	100
14	"	"	"	204	"	0.1 cc. of 7/200 sol. (light ppt.).	(?)	"	204	100
15	"	"	"	139	"	"	(?)	"	37	26
Experiment No. 184. 8, 3, '33. Silver nitrate. Phallusia.										
1	6 × 1.8 cm.	9 cc.	30 min.	196	Sea-water.	Control.	8.4	6 hrs. (1:40–7:40 p.m.).	41	20
2	"	"	"	246	"	"	"	"	19	7.7
3	"	"	"	196	"	"	"	"	9	4.5
8	"	"	"	213	$AgNO_3$.	0.1 cc. of M/100 sol. (light ppt.).	"	"	Killed	0
9	"	"	"	197	"	"	"	"	"	0
10	"	"	"	224	"	0.02 cc. of M/100 sol. (light ppt.).	"	"	102	45
11	"	"	"	207	"	"	"	"	Killed	0
Experiment No. 187b. 8, 4, '33. Sodium chloride. Polyandrocarpa.										
1	8 × 2.5 cm.	27 cc.	8–30 min.	31	Sea-water.	Control.	8.4	7 hrs., 39 min.	0	0
7	"	"	"	37	NaCl.	Final concen.: 0.0127 M.	"	7 hrs., 22 min. (9:48–5:10 p.m.).	0	0
9	"	"	"	50	Sea-water.	Control.	"	7 hrs., 18 min.	0	0
Experiment No. 187c. 8, 4, '33. Potassium chloride. Polyandrocarpa.										
1	8 × 2.5 cm.	27 cc.	8–30 min.	31	Sea-water.	Control.	8.4	7 hrs., 39 min.	0	0
8	"	"	"	45	KCl.	Final concen.: 0.0127 M.	"	7 hrs., 21 min. (9:49–5:10 p.m.).	0	0
9	"	"	"	50	Sea-water.	Control.	"	7 hrs., 18 min.	0	0

TABLE 15—*Experiment No. 159: 7, 21, '33. HCL. Phallusia.*

Number of vial	Size of vial	Capacity of vial	Age of larvæ	Number of larvæ	Medium		pH.	Duration of experiment	Metamorphosed larvæ	
									Number	Per cent
1	6 × 1.8 cm.	9 cc.	90 min.	41	Sea-water.	Control.	8.4	6 hrs. (2:20–8:20).	3	7.3
2	"	"	"	90	"	"	"	"	2	2.2
3	"	"	"	68	"	"	"	"	10	14.7
4	"	"	"	89	Hcl.	Corked after mixing until used.	6.9	"	4	4.4
5	"	"	"	104	"	"	"	"	6	5.7
6	"	"	"	86	"	"	"	"	4	4.6
7	"	"	"	91	"	Air bubbled through 12 hrs. before used.	8.2	"	0	0
8	"	"	"	93	"	"	"	"	1	1
9	"	"	"	96	"	"	"	"	0	0

$BaCl_2$ are added to sea-water. In some experiments the quantities of these reagents used is very close to that required to produce a precipitate; slightly less in some, slightly more in others. These form a graded series in the effect produced: (1st) when no precipitate is formed; (2d) when it forms immediately after the vials are stocked with larvæ and (3d) when it appears at various time-intervals during the experiment.

Invariably the occurrence of metamorphosis coincides with the coming or presence of the precipitate. If no precipitate appears, the percentage of metamorphosis is that of the control groups of larvæ in normal sea-water. If a precipitate comes one or two hours after a vial has been stocked with larvæ, metamorphosis is correspondingly delayed. After a precipitate begins to appear, the larvæ begin almost immediately to attach to the side of the vial and the disruptive phase of metamorphosis follows in an epidemic, usually within a half hour, and often metamorphosis of an entire group has occurred in less than two hours.

When these precipitate-forming substances are added to sea-water several hours in advance of setting an experiment and the precipitate is removed before use, the filtrate has no effect in inducing metamorphosis. If, however, the precipitate is permitted to settle out and sink to the bottom of the vials, metamorphosis occurs apparently in proportion to the extent that the larvæ come into contact with it, as examples: in experiment 155 vials 7, 8 and 9 (table 11) an average of 9.2 per cent metamorphosis occurred as compared with 7.0 per cent in the control (vials 16, 17 and 18), while in experiment No. 187 (table 12) an average of 88 per cent took place in vials 3 and 4, containing the settled precipitate, compared with none in the control (vials 1 and 9).

In all experiments with other media or methods of physical stimulation, metamorphosis has occurred only after the negative response to light and the positive response to gravity have appeared in the larvæ; but, in the presence of these precipitates, attachment and metamorphosis often occur when the larvæ are still oriented at the most illuminated region at the top of the vials. It is as if some substance in the precipitate acted directly on the nervous system of the larvæ in disregard of both susceptibility substance and the by-product of activity.

The precipitates produced by the addition of NaOH and KOH at these pH values consist mainly of $Mg(OH)_2$ (A. R. Haas 1916). Although not analyzed, it may be inferred from a study of the solubility coefficients of barium salts that the precipitate formed with $BaCl_2$ consists of a mixture of barium carbonate and a little barium sulphate. The specific substance involved in the acceleration of metamorphosis is not known.

In another experiment in which the pH was lowered by equilibrating the sea-water used by bubbling through controlled mixtures of CO_2 and air for 16 hours, the resulting pH values were 6.9 and 5.7, which did not change during the experiment. Air was bubbled through the sea-water used in the

controls for a similiar period, the pH remaining at 8.4. When the larvæ of this experiment were killed after six hours of treatment, the control showed over 95 per cent metamorphosis, while the media of pH 6.9 showed only 45 per cent and the larvæ in media pH 5.7 were all dead. This is in direct opposition to results obtained by Berrill (1929, page 60), who found metamorphosis to be increased by increasing partial pressure of CO_2, thus lowering the pH.

LACTIC ACID

On the assumption that lactic acid is formed in muscle tissue of a swimming larva and that its formation or action may be one of the events linked in the process of metamorphosis, six experiments were carried out in which groups of larvæ were subjected to solutions of lactic acid of various concentration, prepared as follows:

Concentrated lactic acid, 85 to 90 per cent (Baker and Adamson), was diluted 1:500 with distilled water. This served as a stock solution. Immediately before use 0.2, 0.4, 0.6, 0.8 or 1.2 cc. of this stock solution was diluted to a volume of 25 cc. by the addition of sea-water. In setting up an experiment with *Phallusia* larvæ, shell vials having a capacity of 9 cc. were used, to which in series 7 1/3 cc. of each of these solutions was added, then 1 2/3 cc. of sea-water and larvæ. With *Polyandrocarpa* larvæ, vials of 27 cc. capacity received 22 cc. of the lactic acid media and 7 cc. of sea-water with the larvæ. The graded pH values of these lactic acid media when first mixed and after standing 6 hours are shown in the following table:

Stock solution	Experimental pH values	
	at beginning	after 6 hrs.
0.2 cc	8.4	8.4
0.4	8.2	8.2
0.6	7.8	7.9
0.8	7.6	7.8
1.2	6.8	7.1

The detail arrangements in each of these experiments and the results in terms of the number and percentage of metamorphosis that occurred in the treated and control groups of larvæ are shown in tables 16 to 18.

These results indicate that concentrations of lactic acid to be effective in causing a significant percentage of metamorphosis should be greater than 1 to 10000, considering 1 cc. of the 85 to 90 per cent solution of lactic acid as unity. This concentration seems much higher than that likely to be produced in the tissues of a larva by its swimming but the degree of perme-

TABLE 16—*Experiment No. 133: 7, 5, '33. Lactic acid. Phallusia larvæ.*

Number of vial	Size of vial	Capacity of vial	Age of larvæ	Number of larvæ	Medium	Concentration	pH.	Duration of experiment	Metamorphosed larvæ	
									Number	Per cent
1	6 × 1.8 cm.	9 cc.	50 min.	147	Lactic acid.	1–9000 cc.	7.9	14 hrs., 40 min. (2:50 p.m.–5:30 a.m.).	76	59
2	"	"	"	179	" "	"	7.9	"	123	68.7
3	"	"	"	151	" "	"	7.9	"	72	40
4	"	"	"	161	Sea-water.	Control.	8.4	"	4	2.5
5	"	"	"	169	"	"	8.4	"	14	8.3
6	"	"	"	174	"	"	8.4	"	11	6.3
Experiment No. 134: 7, 5, '33. Lactic acid. Phallusia larvæ,										
1	6 × 1.8 cm.	9 cc.	1½ hrs.	69	Lactic acid.	1–12500 cc.	7.9	14 hrs. (3:30 p.m.–5:30 a.m.).	49	71
2	"	"	"	91	" "	"	7.9	"	78	82
3	"	"	"	93	" "	"	7.9	"	80	86
4	"	"	"	67	Sea-water.	Control.	8.4	"	14	20
5	"	"	"	115	"	"	8.4	"	32	27
6	"	"	"	82	"	"	8.4	"	22	26

TABLE 17—*Experiment No. 140: 7, 9, '33. Lactic acid. Phallusia larvæ.*

Number of vial	Size of vial	Capacity of vial	Age of larvæ	Number of larvæ	Medium	Concentration	pH.	Duration of experiment	Metamorphosed larvæ	
									Number	Per cent
1	6 × 1.8 cm.	9 cc.	40 min.	119	Lactic acid.	1–62500 cc.	8.4	6 hrs. (2:40–8:40 p.m.).	16	13.4
2	"	"	"	118	" "	"	8.4	"	13	11
3	"	"	"	134	" "	"	8.4	"	65	48
4	"	"	"	132	" "	1–31250 cc.	8.2	"	20	15
5	"	"	"	124	" "	"	8.2	"	21	16.8
6	"	"	"	252	" "	"	8.2	"	44	17.4
7	"	"	"	137	" "	1–20833 cc.	7.8	"	31	22.6
8	"	"	"	150	" "	"	7.8	"	23	15.3
9	"	"	"	125	" "	"	7.8	"	36	28.8
10	"	"	"	109	" "	1–15625 cc.	7.6–8	"	30	27.5
11	"	"	"	122	" "	"	7.6–8	"	58	47.5
12	"	"	"	223	" "	1–10416 cc.	7.5–7.8	"	127	56.9
13	"	"	"	226	" "	"	7.5–7.8	"	112	50
14	"	"	"	127	Sea-water.	Control.	8.4	"	40	31.5
15	"	"	"	131	"	"	8.4	"	15	11.4
16	"	"	"	112	"	"	8.4	"	19	17
Experiment No. 160: 7, 22, '33. Lactic acid. Phallusia larvæ.										
1	6 × 1.8 cm.	9 cc.	30 min.	177	Lactic acid.	1–10416 (vials covered).	6.8–7.7	8 hrs. (2:00–10:00 p.m.).	57	32
2	"	"	"	143	" "	" "	"	"	76	53
3	"	"	"	198	" "	" "	"	"	40	20
4	"	"	"	158	Sea-water.	Control.	8.4	"	4	2.5
5	"	"	"	146	"	"	8.4	"	12	8.2
6	"	"	"	129	"	"	8.4	"	23	17.9

TABLE 18—*Experiment No. 166: 7, 25, '33. Lactic acid. Phallusia larvæ.*

Number of vial	Size of vial	Capacity of vial	Age of larvæ	Number of larvæ	Medium	Concentration	pH.	Duration of experiment	Metamorphosed larvæ	
									Number	Per cent
1	6 × 1.8 cm.	9 cc.	50 min.	190	Lactic acid.	1–9000 cc.	6.7	6 hrs., 35 min.	28	14.7
2	"	"	"	139	" "	"	"	"	18	12.9
3	"	"	"	184	" "	"	"	"	94	51
4	"	"	"	185	Sea-water.	Control.	8.4	"	16	9.1
5	"	"	"	194	" "	"	"	"	4	2
6	"	"	"	196	" "	"	"	"	25	12.7
Experiment No. 165: 7, 24, '33. Lactic acid. Polyandrocarpa larvæ.										
1	8 × 2.5 cm.	27 cc.	15 min.	62	Lactic acid.	1–9000 cc.	6.8–7.2	4¼ hrs. (9:45–2:00).	47*	75
2	"	"	"	68	" "	"	6.8–7.1	4⅕ hrs. (9:48–2:00).	61**	89
3	"	"	"	33	Sea-water.	Control.	8.4	4⅛ hrs. (9:40–2:00).	1	3
4	"	"	"	73	"	"	8.4	4⅙ hrs. (9:50–2:00).	2	2.7

* 8 larvæ metamorphosed at 11:30; 21 at 12:30; 45 at 1:00 p.m.; 47 at 2 p.m.
** 10 larvæ metamorphosed at 11:30; 37 at 12:30; 44 at 1:00 p.m.; and 61 at 2:00 p.m.

ability of the larva to lactic acid in the sea-water is an important unknown factor. The extreme slowness of its accelerating effect as compared with other accelerating agents would seem to remove lactic acid from consideration as a factor in the normal process of metamorphosis.

The assumption that the low pH of the lactic acid media may be an important factor in the acceleration of metamorphosis would agree with the conclusion reached by Dr. Berrill (1929), elsewhere stated, but it is rejected because of the many unambiguous results of other experiments, to be described later, that show the pH of the medium to be a negligible factor. The acceleration of metamorphosis occurring in these experiments is assumed to be due to an effect of the lactate radical.

ACETYL CHOLINE, ADRENALIN AND PITUITRIN "O"

Larvæ were subjected to sea-water solutions of the substances listed in the sub-title above,[1] but the experiments carried out were few and inconclusive in result and will be referred to briefly only as a record of work begun. The proper concentration of each substance may be arrived at only by repeated trials and the time available for this work was too short.

ACETYL CHOLINE

In one experiment of 6 hours duration (July 20), with acetyl choline in concentrations of 1 x 10^6 and 5 x 10^7, metamorphosis was inhibited as indicated by 4.4 and 3.6 per cent, respectively, in comparison with 22.6 per cent metamorphosis in the control groups of *Phallusia* larvæ. In a second experiment of 8 hours duration (July 22) a concentration of 1 x 10^6 gave 7.9 per cent metamorphosis, the control vials showing an average of 9 per cent.

ADRENALIN

One experiment only was made with adrenalin (July 26), the concentrations being 1 x 10^6 and 2 x 10^6. In the former medium the larvæ soon became inactive, as if narcotized, and no metamorphosis had occurred after 16 hours. In the latter, 4.3 per cent metamorphosis took place as compared with 34 per cent in the control vials. This inhibition of metamorphosis may be accounted for on the assumption that swimming activity was prevented by the narcotizing effect of something in the medium, not necessarily the hormone adrenalin.

PITUITRIN "O"

In the single experiment with the hormone, pituitrin "O," all larvæ were almost instantly killed. The 0.5 cc. of the substance in an ampoule was diluted to 22 cc. of sea-water and 7⅓ cc. of this solution, with 1⅔ cc. of sea-water plus larvæ, were added to each of three vials. The lethal effect

[1] The adrenalin and pituitrin "O" that were used are the commercial products of Parke Davis Company; the acetylcholine was prepared by Eastman Kodak Company.

was not necessarily due to the part of the medium represented by pituitrin "O."

PITUITARY WHOLE GLAND, AND ANTERIOR LOBE

Sea-water solutions of the substances listed in the sub-title were made up for use in experiments as follows: 500 mg. of the dry powder, as prepared by the Parke Davis Company, was ground ten minutes with mortar and pestle in 10 cc. of sea-water, filtered through Whatman's No. 40 filter paper, and the residue rinsed with an additional 10 cc. of sea-water. The final experimental media for *Phallusia* larvæ consisted of 0.5 cc. of these combined filtrates diluted with sea-water plus larvæ to a volume of 9 cc., or for *Polyandrocarpa* larvæ, of 1.5 cc. of the filtrate diluted with sea-water plus larvæ to a volume of 27 cc., each having a pH of 8.4.

The results of all experiments with these whole-gland extracts, including that of whole thyroid gland, were so decisive in the large percentage of metamorphosis induced, as compared with that occurring in the control groups of larvæ, that it seemed unnecessary to make many repetitions. Two experiments with all essential details are summarized in table 19.

In one of these experiments, No. 174, the relative effectiveness of three whole-gland preparations with groups of larvæ of the same age and parentage is shown.

The ineffectiveness of purified hormone preparations, just described, in accelerating the process of metamorphosis is in striking contrast with the effectiveness of the several whole-gland extracts. This suggests that some tissue substance, possibly some soluble intracellular ferment, common to all of these whole-gland preparations, is the agent of acceleration rather than a common effect of the several hormones themselves.

THYROID GLAND EXTRACT

Despite the fact that the processes of metamorphosis in larval forms of amphibians and ascidians have almost nothing in common but the name, the structural changes in the two cases being entirely different, the known dependence of the former upon the action of the hormone, thyroxin, secreted by the thyroid gland, is a sufficient reason for an experimental inquiry concerning the rôle this substance may play in ascidian metamorphosis.

For this work desiccated thyroid gland, as prepared by Parke Davis and Company, was provided and extracts were made, as follows: 0.6 gram (approximately 10 grains) was ground 10 minutes with mortar and pestle in 10 cc. of sea-water. This solution was filtered (Whatman's No. 40) and the residue washed with an additional 10 cc. of sea-water. The combined filtrate of about 18 cc. was used in making experimental media, 1 cc.-0.005 cc. of the filtrate being added to sea-water (plus larvæ) to make a final volume of 9 cc. for each experiment with larvæ of *Phallusia nigra*, or, in the same ratio, to make a volume of 27 cc. for experiments with larvæ of

TABLE 19—*Experiment No. 174: 7, 29, '33. Pituitary whole gland and anterior lobe. Thyroid whole gland. Phallusia.*

Number of vial	Size of vial	Capacity of vial	Age of larvæ	Number of larvæ	Medium	Concentration	pH.	Duration of experiment	Metamorphosed larvæ	
									Number	Per cent
1	6 × 1.8 cm.	9 cc.	30 min.	191	Sea-water.	Control.	8.4	4 hrs. (2:00–6:00 p.m.).	0	0
2	"	"	"	200	"	"	"	"	6	3
3	"	"	"	164	"	"	"	"	19	11.5
4	"	"	"	146	Pituitary whole gland extract.	.5 cc.	"	"	103	70
5	"	"	"	202	"	.5 cc.	"	"	163	80
6	"	"	"	193	"	1.0 cc.	"	"	190	98
7	"	"	"	233	Anterior lobe extract.	.5 cc.	"	"	219	93
8	"	"	"	202	"	.5 cc.	"	"	190	94
9	"	"	"	209	"	1.0 cc.	"	"	187	89
10	"	"	"	253	Thyroid whole gland extract.	.5 cc.	"	"	167	66
11	"	"	"	182	"	.5 cc.	"	"	108	60
12	"	"	"	164	"	1.0 cc.	"	"	99	60
Experiment No. 183: 8, 2, '33. Anterior lobe. Polyandrocarpa.										
1	8 × 2.5 cm.	27 cc.	7–30 min.	46	Sea-water.	Control.	8.4	4 hrs., 23 min. (10:22–2:45 p.m.).	0	0
6	"	"	"	70	Anterior lobe extract.	1.5 cc.	"	4 hrs., 3 min. (10:40–2:45).	40	57
7	"	"	"	56	Sea-water.	Control.	"	4 hrs. (10:45–2:45 p.m.).	0	0

Polyandrocarpa tincta. The final pH value of this medium was 8.4, that of normal sea-water at the Tortugas.

Extracts prepared in this way were used in ten experiments with *Phallusia* larvæ, two with the larva of *Polyandrocarpa.* In two of the former an amount of NaOH solution was added to the thyroid gland extract to give the medium a pH value of 9.0 to 9.1. In one experiment with each of these larvæ, sea-water solutions of NaCN, neutralized to a pH of 8.4, were used to dilute the thyroid gland extract.

Tables follow giving the details of the arrangement, duration and results of five of these experiments.

It may be stated that thyroid gland extract consistently induced a significant percentage of metamorphosis in experiments in which the concentrations used were obtained by diluting quantities of the filtrate varying between 0.5 cc. and 0.1 cc., to a volume of 9 cc. The concentration produced by 1 cc. in this dilution was slightly toxic to the larvæ, and concentrations obtained by the use of less than 0.1 cc. of the stock solution per vial showed a diminution in the effect. The effectiveness of the thyroid gland extract seems to be consistently increased by the addition of an amount of NaOH solution sufficient to give the medium a pH value of about 9. This effect is strikingly shown in Experiment No. 151, but seems to be inhibited by NaCN in concentrations of $\frac{M}{30000}$ and $\frac{M}{45000}$, experiment No. 180. The inhibitory effect is greatly diminished in a $\frac{M}{90000}$ solution. The number of experiments with cyanide is too few, however, to afford a basis for a valid conclusion.

P. Weiss (1928), using a thyroid gland extract in sea-water in which large groups of the larva of *Ciona intestinalis* remained from 1 to 6 hours, obtained a consistently larger number of cases of metamorphosis in treated than in controls and concluded that the vertebrate thyroid hormone, necessary for metamorphosis of amphibian tadpoles, is also effective in inducing metamorphosis in larvæ of ascidians. In the experiments just described, much larger percentages of metamorphosis were obtained than occurred in those reported by Dr. Weiss, but in neither case is there proof that the effective agent is the hormone, thyroxin. Many substances other than thyroxin are present in a thyroid whole-gland preparation. The equal effectiveness of other gland and tissue extracts in causing metamorphosis, elsewhere described, especially extracts of mantle and atrial tissues taken from adult ascidians, indicates that thyroxin is probably not the specific agent causing the metamorphosis in any of these experiments; more probably the activating substance is some soluble intracellular ferment. It is unfortunate for the settlement of this interesting question that thyroxin itself was not available for trial.

TABLE 20—*Experiment No. 148: 7, 13, '33. Thyroid gland extract. Phallusia larvæ.*

Number of vial	Size of vial	Capacity of vial	Age of larvæ	Number of larvæ	Medium	Concentration	pH.	Duration of experiment	Metamorphosed larvæ	
									Number	Per cent
1	6 × 1.8 cm.	9 cc.	20 min.	241	Thyroid gland extract.	1 cc.	8.4	4 hrs. (2–6 p.m.).	181	75
2	"	"	"	216	"	"	"	"	95	44
3	"	"	"	187	"	"	"	"	138	73
4	"	"	"	198	"	.05 cc.	"	"	111	55
5	"	"	"	157	"	"	"	"	109	63
6	"	"	"	155	"	"	"	"	115	74
7	"	"	"	181	"	.25 cc.	"	"	132	72
8	"	"	"	195	"	"	"	"	26	13
9	"	"	"	153	"	"	"	"	93	60
10	"	"	"	164	"	.1 cc.	"	"	78	47
11	"	"	"	160	"	"	"	"	67	42
12	"	"	"	189	"	"	"	"	93	49
13	"	"	"	146	"	.02 cc.	"	"	25	17
14	"	"	"	182	"	"	"	"	2	1
15	"	"	"	173	"	"	"	"	52	30
16	"	"	"	186	"	.005 cc.	"	"	53	28
17	"	"	"	184	"	"	"	"	43	23
18	"	"	"	163	"	"	"	"	12	7
19	"	"	"	175	Sea-water.	Control.	"	"	9	5
20	"	"	"	175	"	"	"	"	12	6.8
21	"	"	"	163	"	"	"	"	19	9.5

TABLE 21—*Experiment No. 151: 7, 16, '33. Thyroid extract and NaOH. Phallusia larvæ.*

Number of vial	Size of vial	Capacity of vial	Age of larvæ	Number of larvæ	Medium	Concentration	pH.	Duration of experiment	Metamorphosed larvæ	
									Number	Per cent
1	6 × 1.8 cm.	9 cc.	25 min.	126	Thyroid extract.	0.5 cc.	8.4	4½ hrs. (2:15–6:45 p.m.).	50	39
2	"	"	"	152	" "	"	8.4	"	58	37
3	"	"	"	119	" "	"	8.4	"	22	20
4	"	"	"	127	" "	" and NaOH.	9.1	3⅓ hrs. (2:15–5:35 p.m.).	103	81
5	"	"	"	150	" "	" " "	9.1	"	103	68
6	"	"	"	111	" "	" " "	9.1	"	98	88
7	"	"	"	156	Sea-water.	Control.	8.4	4½ hrs. (2:15–6:45 p.m.).	0	0
8	"	"	"	128	"	"	8.4	"	0	0
9	"	"	"	141	"	"	8.4	"	0	0
Experiment No. 180: 8, 1, '22. Thyroid gland extract and cyanide. Phallusia larvæ.										
1	6 × 1.8 cm.	9 cc.	30 min.	113	Thyroid extract.	0.5 cc.	8.4	4½ hrs. (2:20–6:50 p.m.).	78	69
2	"	"	"	100	" "	0.5 cc.	"	"	65	65
3	"	"	"	121	Thyroid extract and NaCN	0.5cc.+M/30000	"	"	21	17
4	"	"	"	107	"	"	"	"	10	9
5	"	"	"	109	"	0.5cc.+M/45000	"	"	32	29
6	"	"	"	115	"	"	"	"	29	25
7	"	"	"	80	"	0.5cc.M+/90000	"	"	44	55
8	"	"	"	98	"	"	"	"	53	54
9	"	"	"	110	NaCN	M/90000	"	"	3	2.7
10	"	"	"	94	Sea-water.	Control.	"	"	1	1
11	"	"	"	101	"	"	"	"	1	1

TABLE 22—*Experiment No. 157: 7, 20, '33. Thyroid gland extract. Polyandrocarpa larvæ.*

Number of vial	Size of vial	Capacity of vial	Age of larvæ	Number of larvæ	Medium	Concentration	pH.	Duration of experiment	Metamorphosed larvæ	
									Number	Per cent
1	8 × 2.5 cm.	27 cc.	10 min.	69	Thyroid extract.	3.0 cc.	8.4	(10:25 a.m.–3:30 p.m.).	68*	99
2	"	"	15 "	52	" "	1.5 cc.	"	(10:30 a.m.–3:30 p.m.).	52**	100
3	"	"	5 "	84	Sea-water.	Control.	"	(10:20 a.m.–3:30 p.m.).	15	17.8
4	"	"	30 "	87	"	"	"	(10:45 a.m.–3:30 p.m.).	4	4.5
Experiment No. 178: 7, 31, '33. Thyroid gland extract and cyanide. Polyandrocarpa larvæ,										
1	8 × 2.5 cm.	27 cc.	5 min.	57	Thyroid extract.	1.5 cc.	8.4	4 hrs. (12:35–4:35).	57****	100
2	"	"	"	63	" "	1.5 and 0.002M. cyanide.	"	"	***	
3	"	"	"	72	Cyanide	.002M.	"	"	***	
4	"	"	"	26	Sea-water.	Control.	"	"	0	0
5	"	"	"	112	"	"	"	"	0	0

* All but 1 metamorphosed at 1:30 p.m.
** All metamorphosed at 1:30 p.m.
*** All larvæ killed almost immediately.
**** All but one larva metamorphosed at 3:30 p.m. (17 at 1:20 p.m.; 19 others at 2:00 p.m.; 10 others at 3:00 p.m.).

E. A. Spaul (1928) carried out two experiments in which separate lots of tadpoles of *R. temporaria* were fed with fresh endostyle, pharynx and mantle tissues of the ascidian, *Ciona intestinalis*, none of which showed any accelerating effect on metamorphosis, while the control sets of tadpoles, 8 days after feeding on dog-fish thyroid, showed well-developed hind limbs. He concluded that the ascidian endostyle secretes no principle to be identified by its effect upon amphibian metamorphosis.

FRESH TISSUE EXTRACTS AND BERKSHIRE SAND

An extract of the vertebrate thyroid gland having induced a significant percentage of metamorphosis in both species of ascidian larvæ, questions were raised; whether the observed acceleration of metamorphosis is due to the thyroxin content of the extract; whether metamorphosis will be induced by an extract of the ascidian homologue of the thyroid gland, the endostyle; and whether the developing endostyle in the larva secretes the thyroxin necessary for metamorphosis of the larva.

An answer to the second of these questions was sought by subjecting groups of larvæ to extracts of ascidian endostyles prepared as follows: endostyles were dissected from a considerable number of adults of *Phallusia nigra,* 17 in the first instance, care being taken that the adjacent tissue of the pharynx was not included. The endostyles, partially dried by pressing them between layers of filter paper, weighed (211.2 mg). Ground 15 minutes in a mortar with 5 cc. of sea-water, filtered through Whatman's No. 40 filter paper, the residue rinsed with 5 cc. of additional sea-water, yielded about 8 cc. of a clear purplish liquid extract, the color probably derived from pigment cells in the endostyle tissue. It is important to state that a quantity of Berkshire sand obtained through the A. H. Thomas Co. was placed in the mortar with the endostyles in order to facilitate the grinding process and insure complete maceration of the tissue cells.

In the experiment in which this extract was used, 5 cc. in one vial, 3 cc. in another, 98 and 80 per cent metamorphosis, respectively, occurred in 4½ hours, 10 per cent only having taken place in the control groups of *Phallusia* larvæ.

For a second experiment, the endostyle extract was prepared in the same way except that the mixture of endostyle tissue and Berkshire sand was washed in the filter paper a second time with 10 cc. of sea-water, the washings kept separate from the extract proper. 4 cc. and 3 cc., respectively, of the more concentrated extract were placed in two vials and diluted with sea-water plus larvæ to volumes of 9 cc. The washings (7⅓ cc.) were placed in a third vial and diluted with sea-water plus larvæ to the same volume. The control consisted of groups of larvæ of the same lot in three vials of the same capacity. This experiment covered a period of 2 hours 50 minutes. No metamorphosis occurred in either of the control vials; 99

and 96 per cent, respectively, in the vials containing the extract proper, 100 per cent in the vial containing the endostyle and sand washings.

In the enthusiasm generated by the remarkable results of the experiments just described, thyroxin was specifically visualized as constituting the "susceptibility" substance of the mechanism of metamorphosis, but, to avoid premature conclusion that the endostyle provides the hormone required for the metamorphosis of the larva, a third lot of extracts were again prepared, including extracts of mantle, and atrial tissue as well as that of endostyles, Berkshire sand being used again in each case in the grinding process, as follows: endostyle (plus sand) extract, 15 cc.; endostyle (plus sand) washings, 10 cc.; mantle tissue (plus sand) extract, 9 cc.; mantle tissue (plus sand) washings, 9 cc.; atrial tissue (plus sand) extract, 15 cc. The results of the third of these experiments (Number 171), carried out July 27, are presented in table 23.

Extracts of mantle and atrial tissues were thus shown to be as effective in accelerating metamorphosis as endostyle extract. The conclusion that thyroxin may be the accelerating agent in former experiments, not only with endostyle extracts but also with an extract of the thyroid gland of vertebrates, was shown to be untenable:

Groups of larvæ were now treated with extracts prepared from other tissues; *Ptychodera*, fish liver (*Lutianus griseus*), mantle tissue of a species of ascidian belonging to the genus *Cynthia*, all extracts prepared in the same way with Berkshire sand, and all yielding a high percentage of metamorphosis; *Ptychodera* tissue extract, 74 per cent fish liver, 96 per cent *Cynthia* mantle extract, 94 per cent, *Phallusia* mantle extract (left over from the previous experiment), 97 per cent, the control vials averaging 3 per cent.

The results were bewildering. Some plausible explanation for the cause of the universal and practically equal potency of these diverse extracts was sought for. Berkshire sand had been used in the preparation of all of these fresh tissue extracts. Could this insoluble silicon dioxide have contributed the accelerating agent to these extracts?

It was now necessary that all tissue extracts should be prepared without the use of sand and tested again and that an investigation be made of the Berkshire sand to ascertain if possible whether it contained something potent in accelerating metamorphosis.

Endostyles and mantle-atrium tissues were again dissected, dried, weighed and thoroughly ground in sea-water, no sand being used, the filtrate having the same clear purplish appearance as before. A sea-water extract of Berkshire sand was also prepared by grinding a quantity of the sand (about 3 teaspoonfuls for 10 minutes) in 10 cc. of sea-water. These filtrates were used in making the experimental media for experiments 174 with larvæ of *Phallusia nigra*, and 175 with larvæ of *Polyandrocarpa tincta*. These experiments, shown in table 23, demonstrated that Berkshire sand gives

TABLE 23—*Experiment No. 171: 7, 27, '33. Phallusia endostyle atrium and mantle extracts. Phallusial arvæ.*

Number of vial	Size of vial	Capacity of vial	Age of larvæ	Number of larvæ	Medium	Concentration	pH.	Duration of experiment	Metamorphosed larvæ	
									Number	Per cen
1	6 × 1.8 cm.	9 cc.	25 min.	182	Sea-water.	Control.	8.4	4 hrs. (2:10–6:10 p.m.).	0	0
2	"	"	"	164	"	"	"	"	0	0
3	"	"	"	184	"	"	"	"	3	2
4	"	"	"	163	Endostyle extract.	3 cc.	"	"	142	87
5	"	"	"	154	" "	1.5 cc.	"	"	36	23
6	"	"	"	149	" " washings.	3 cc.	"	"	51	34
7	"	"	"	140	Atrial tissue extract.	5 cc.	"	"	134	95
8	"	"	"	193	" " "	3 cc.	"	"	191	99
9	"	"	"	167	" " " washings.	7⅓ cc.	"	"	167	100
10	"	"	"	168	Mantle tissue extract.	5 cc.	"	"	168	100
11	"	"	"	127	" " "	3 cc.	"	"	127	100
	Experiment No. 174: 7, 29, '33. Phallusia mantle-atrium and Berkshire sand extracts. Phallusia larvæ.									
1	6 × 1.8 cm.	9 cc.	30 min.	191	Sea-water.	Control.	8.4	4 hrs. (2–6 p.m.).	0	0
2	"	"	"	200	"	"	"	"	6	3
3	"	"	"	164	"	"	"	"	19	11.5
13	"	"	"	162	Mantle-atrium ext. ground with sand.	3.2 cc.	"	"	162	100
14	"	"	"	165	Mantle-atrium ext. ground without sand.	2 cc.	"	"	165	100
15	"	"	"	121	Sea-water ground with sand.	5 cc.	"	"	116	95
16	"	"	"	167	" " " "	5 cc.	"	"	150	89
	Experiment No. 175: 7, 29, '33. Phallusia mantle-atrium and Berkshire sand extracts. Polyandrocarpa larvæ.									
1	8 × 2.5 cm.	27 cc.	5–18 min.	58	Sea-water.	Control.	8.4	6 hrs., 50 min. (10:10–5 p.m.).	7	12
2	"	"	"	68	Phallusia M-A exts. prepared without sand.	10 cc.	"	6 hrs., 45 min. (10:15–4 p.m.).	6	8
5	"	"	"	93	Phallusia M-A exts. prepared with sand.	10 cc.	"	6⅓ hrs. (10:20–5 p.m.).	90	96
6	"	"	"	72	Sea-water.	Control.	"	6 hrs., 37 min. (10:23–5 p.m.).	3	4

something to sea-water that greatly accelerates metamorphosis of larvæ of both species and that an extract of *Phallusia* mantle and atrial tissues is remarkably effective in inducing metamorphosis in *Phallusia* larvæ but has no accelerating effect on larvæ of *Polyandrocarpa*. A quantity of Berkshire sand was now subjected to a rigorous cleaning process in order to ascertain whether it contains some impurity that may be responsible for the effect which its sea-water extract has in accelerating metamorphosis. It was first heated to redness, then boiled with strong HCl, and thoroughly washed with distilled water. Three teaspoonfuls of this cleaned sand were ground 10 minutes in 10 cc. of sea-water, the filtrate used in making an experimental medium which, with extracts made from *Phallusia* endostyles and mantle atrium tissues, was tested on larvæ of *Phallusia nigra* in Experiment 176, shown in table 24.

The rigorous cleaning treatment given the Berkshire sand reduced the potency of its sea-water extract about 50 per cent, but it still yielded something to sea-water having the property of greatly accelerating metamorphosis which was not further eliminated by boiling in aqua regia.[1] This experiment, and others to be discussed later, showed conclusively that the *Phallusia* endostyle and mantle-atrium extracts did not acquire from Berkshire sand their property of accelerating metamorphosis of *Phallusia* larvæ, but that these tissues contain the accelerating substance.

An attempt was made August 1 to approximate more closely to the nature of the accelerating property of *Phallusia* mantle-atrium extract: 1085 mg. of mantle-atrial tissue was ground to a pulp to which 10 cc. of absolute alcohol was added and ground 10 minutes. The filtrate was evaporated to dryness, the residue then taken up with 10 cc. of sea-water which was again filtered, yielding a clear colorless liquid containing the alcohol soluble fraction. The original residue was dried, ground again with sea-water and filtered, the filtrate containing the water soluble fraction and having a purplish color. An experiment (No. 179, shown in table 24) with these fractions showed that both have the property of accelerating metamorphosis of *Phallusia* larvæ, the water soluble fraction, 100 per cent, the alcohol fraction, 84 per cent, as compared with an average of 9 per cent in the control groups of larvæ.

Further experiments with ascidian tissue extracts, of which Nos. 187, 190, 191, 192 and 193 have been selected for tabular description (tables 25 and 26), conclusively show that these extracts are highly specific in their action; *Phallusia nigra* mantle-atrium extract having a slight accelerating effect only on larvæ of *Polyandrocarpa tincta* but inducing 100 per cent metamorphosis consistently in larvæ of the same species. An extract made

[1] Since this work was done, a chemical analysis of sea-water extracts of this Berkshire sand has shown a high percentage of colloidal silicate. It is therefore possible that the acceleration of metamorphosis that takes place in groups of larvæ subjected to media prepared by grinding Berkshire sand in sea-water is due to this colloidal silicate, but no other than a physical effect seems possible.

TABLE 24—*Experiment No. 176: 7, 30, '33. Phallusia endostyle, mantle-atrium extracts and Berkshire sand. Phallusia larvæ.*

Number of vial	Size of vial	Capacity of vial	Age of larvæ	Number of larvæ	Medium	Concentration	pH.	Duration of experiment	Metamorphosed larvæ	
									Number	Per cent
1	6 × 1.8 cm.	9 cc.	25 min.	240	Sea-water.	Control.	8.4	3½ hrs. (2–5:30 p.m.).	44	18
2	"	"	"	164	"	"	"	"	24	14.6
3	"	"	"	190	"	"	"	"	18	9.4
4	"	"	"	212	Endostyle extract.	5 cc.	"	"	212	100
5	"	"	"	176	" "	3 cc.	"	"	176	100
6	"	"	"	191	" " washings.	3.5 cc.	"	"	115	60
7	"	"	"	209	" " "	3.5 cc.	"	"	119	56
8	"	"	"	194	Mantle-atrium extract.	4 cc.	"	"	194	100
9	"	"	"	241	" " "	3 cc.	"	"	241	100
10	"	"	"	237	Berkshire sand extract (cleaned).	4 cc.	"	"	88	37
11	"	"	"	205	"	4 cc.	"	"	102	50
Experiment No. 179: 8, 1, '33. Alcohol and water soluble fractions, phallusia mantle-atrium extract. Phallusia larvæ.										
1	6 × 1.8 cm.	9 cc.	20 min.	167	Sea-water.	Control.	8.4	3¾ hrs. (2:15–6 p.m.).	7	4
2	"	"	"	163	"	"	"	"	31	19
3	"	"	"	121	"	"	"	"	5	4
4	"	"	"	204	Alcohol soluble fraction M-A ext. in sea-water.	4 cc.	"	"	143	70
5	"	"	"	176	"	3 cc.	"	"	163	92
6	"	"	"	173	"	2 cc.	"	"	157	90
7	"	"	"	136	Water soluble fraction M-A ext. in sea-water.	4 cc.	"	"	136	100
8	"	"	"	187	"	3 cc.	"	"	187	100
9	"	"	"	151	"	1.5 cc.	"	"	149	98.5

TABLE 25—*Experiment No. 187: 8, 4, '33. Phallusia mantle extract. Polyandrocarpa larvæ.*

Number of vial	Size of vial	Capacity of vial	Age of larvæ	Number of larvæ	Medium	Concentration	pH.	Duration of experiment	Metamorphosed larvæ	
									Number	Per cent
1	8 × 2.5 cm.	27 cc.	8–30 min.	31	Sea-water.	Control.	8.4	7 hrs., 37 min. (9:31–5:10 p.m.).	0	0
6	"	"	"	57	Mantle-atrium extract.	10 cc.	"	7 hrs., 22 min. (9:48–5:10 p.m.).	12	21
9	"	"	"	50	Sea-water.	Control.	"	7 hrs., 18 min. (9:52–5:10 p.m.).	0	0
Experiment No. 191: 8, 6, '33. Snail, cynthia and polyandrocarpa tissues extracts. Polyandrocarpa larvæ.										
1	8 × 2.5 cm.	27 cc.	8–25 min.	48	Sea-water.	Control.	8.4	6 hrs., 7 min. (9:53–4:00).	2	4
2	"	"	"	30	Snail tissue extract (mantle and foot).	6 cc.	"	6 hrs. (10–4 p.m.).	30	100
3	"	"	"	19	Cynthia mantle tissue extract.	6 cc.	"	6 hrs., 10 min. (10:20–4:30 p.m.).	0	0
4	"	"	"	26	Polyandrocarpa entire colony extract.	7 cc.	"	50 min. (10:10–11 a.m.)	26	100
Experiment No. 190: 8, 6, '33. Polyandrocarpa, snail, cynthia and phallusia tissue extracts. Phallusia larvæ.										
1	6 × 1.8 cm.	9 cc.	20 min.	84	Sea-water.	Control.	8.4	3 hrs. (1:50–4:50 p.m.).	1	1
2	"	"	"	83	"	"	"	"	0	0
3	"	"	"	80	"	"	"	"	0	0
4	"	"	"	96	Polyandrocarpa extract.	2.5 cc.	"	"	0	0
5	"	"	"	82	" "	2.5 cc.	"	"	0	0
6	"	"	"	86	Phallusia mantle-atrium ext.	2 cc.	"	"	86	100
7	"	"	"	98	" " " "	2 cc.	"	"	98	100
8	"	"	"	87	Snail mantle and foot extract.	2 cc.	"	"	0	0
9	"	"	"	74	" " " " "	2 cc.	"	"	0	0
10	"	"	"	83	Cynthia mantle extract.	2 cc.	"	"	0	0
11	"	"	"	79	" " "	2 cc.	"	"	0	0

TABLE 26—*Experiment No. 192: 8, 10, '33. Ascidian and fish tissue extracts. Phallusia larvæ.*

Number of vial	Size of vial	Capacity of vial	Age of larvæ	Number of larvæ	Medium	Concentration	pH.	Duration of experiment	Metamorphosed larvæ	
									Number	Per cent
1	6 × 1.8 cm.	9 cc.	30 min.	117	Sea-water.	Control.	8.4	3 hrs. (2:30–5:30 p.m.).	0	0
2	"	"	"	106	"	"	"	"	0	0
3	"	"	"	94	"	"	"	"	0	0
4	"	"	"	108	Polyandrocarpa colony extract.	2 cc.	"	"	1	.93
5	"	"	"	126	" " "	2 cc.	"	"	1	.79
6	"	"	"	90	Phallusia mantle-atrium extract.	2 cc.	"	"	90	100
7	"	"	"	90	"	2 cc.	"	"	90	100
8	"	"	"	101	Fish brain extract.	2 cc.	"	"	6	5.9
9	"	"	"	93	" " "	2 cc.	"	"	3	3.2
10	"	"	"	135	Fish pituitary gland extract.	2 cc.	"	"	1	.7
11	"	"	"	101	" " " "	2 cc.	"	"	1	1
12	"	"	"	93	Fish muscle extract.	2 cc.	"	"	4	4
13	"	"	"	80	" " "	2 cc.	"	"	8	10
14	"	"	"	72	Fish liver extract.	2 cc.	"	"	0	0
15	"	"	"	80	" " "	2 cc.	"	"	0	0
Experiment No. 193: 8, 10, '33. Ascidian and fish tissue extracts. Polyandrocarpa larvæ.										
1	8 × 2.5 cm.	27 cc.	5–30 min.	37	Sea-water.	Control.	8.4	5½ hrs. (11:55–5:30).	0	0
2	"	"	"	65	Fish brain extract.	6 cc.	"	5½ hrs. (11:59–5:30).	3	4.6
3	"	"	"	94	Polyandrocarpa colony extract.	6 cc.	"	42 min. (12:03–12:45).	94	100
4	"	"	"	124	Fish pituitary extract.	6 cc.	"	5½ hrs. (12:06–5:30).	2	1.6
5	"	"	"	149	Fish muscle extract.	6 cc.	"	5 hrs., 21 min. (12:09–5:30).	35	23.4
6	"	"	"	151	Fish liver extract.	6 cc.	"	5 hrs., 18 min. (12:12–5:30).	15	10.5
7	"	"	"	130	Phallusia mantle-atrium extract.	6 cc.	"	5 hrs., 14 min. (12:16–5:30).	23	17.6
8	"	"	"	107	Sea-water.	Control.	"	5 hrs., 11 min. (12:19–5:30).	1	.9

by grinding an entire colony of *Polyandrocarpa tincta* individuals in sea-water, induced 100 per cent metamorphosis in larvæ of the same species but had slight accelerating effect on larvæ of *Phallusia nigra*. An extract made from mantle-atrium tissue of an ascidian belonging to the genus *Cynthia* had very little accelerating effect on larvæ of either *Phallusia nigra* or *Polyandrocarpa tincta*. An extract made from tissue taken from the mantle and foot of a snail exercised a high accelerating effect on larvæ of *Polyandrocarpa* but none on larvæ of *Phallusia*.

It seems hardly possible that the accelerating agents present in extracts of these fresh ascidian tissues can be the same as those in extracts made from whole-gland preparations of vertebrate thyroid gland, pituitary whole-gland or anterior lobe. The former shows a high degree of specificity, the latter none. As a tentative hypothesis, the suggestion may be hazarded that the accelerating agents extracted from fresh ascidian tissues may prove to be endoenzymes, or ferments, each occurring only in a single species of ascidian and catalyzing a specific metabolic reaction, while the accelerating agent extracted from the thyroid and pituitary glands behaves as if it were an endoenzyme of a more general or basic nature, catalyzing a reaction common to a wide range of organisms.

Experiments Nos. 192 and 193 were carried out by Mr. Nicoll to ascertain the effect of extracts of various fish tissues prepared without the use of Berkshire sand. As compared with the 100 per cent acceleration induced by an ascidian tissue extract on larvæ of the same species, the effect of the fish tissue extracts was shown to be negligible, except perhaps in the case of fish-muscle extract. But here the experiments are too few to afford a safe basis for interpretation. If muscle tissue of whatever origin should prove to contain an accelerating substance, an explanation might be afforded for the exceptional result of Experiment 191, in which an extract of mantle and foot tissue of a snail (largely composed of muscle fibers) induced 100 per cent metamorphosis in a group of larvæ of *Polyandrocarpa* in a period of 6 hours, 4 per cent only having occurred in the control vial. It should not be overlooked, however, that the snail extract was very slow in its accelerating effect compared with that of the *Polyandrocarpa* extract, the latter having caused 100 per cent metamorphosis within 50 minutes.

SUMMARY AND CONCLUSIONS

The observations made in course of this investigation are interpreted as follows:

(1) The ascidian larva is a dual organism, the action system of the larva being quite separate from the action system of the ascidiozooid, the former exercising an inhibiting effect on the growth and differentiation of the latter throughout the free-swimming period, the inhibition ceasing upon the occurrence of the disruptive phase of metamorphosis.

(2) Metamorphosis advances by three stages; (a) changes in the adaptive responses of the larva to light and gravity; (b) the attachment of the larva to the surface of some foreign object; (c) the disruptive phase during which the entire larval action system is destroyed.

(3) Swimming activity causes the production and concentration of some metabolic product in the larval tissues that is essential to the induction of metamorphosis.

(4) Swimming is most effectively and consistently caused by subjecting larvæ to a series of sudden changes in light intensity, but under certain conditions, activity may also be induced by mechanical stimuli transmitted to the larvæ by jarring the containing vial or by agitating the water in the vial.

(5) The presence in the larval tissues of metabolic products resulting from swimming is not alone sufficient to induce metamorphosis, but another substance with which this metabolic product may react is equally necessary.

(6) This other substance is apparently produced during the free-swimming period as a secretion by some larval tissue or organ but is extremely variable in time of appearance. It has been designated the "susceptibility substance" or factor.

(7) The great variability of ascidian larvæ of the same species in the duration of their free-swimming period is apparently due to variability in the time of formation of the susceptibility substance and hence to the time of differentiation of the larval organ that produces it.

(8) The free-swimming period of the larva of *Phallusia nigra* varies under laboratory conditions from about 2 hours to more than 5 days; of the larva of *Polyandrocarpa tincta*, from about 2 to 36 hours.

(9) A third substance is formed in a larva by a reaction between the susceptibility substance and some product of swimming activity, which apparently acts directly on the larval nerve centers to cause the progressive series of responses enumerated in (2) that constitute the process of metamorphosis.

(10) Metamorphosis may be induced artificially by diverse chemical and biological substances placed in sea-water with groups of larvæ: lactic acid; precipitates formed in sea-water by the addition of NaOH, KOH and $BaCl_2$; CO_2-free sea-water; pituitary whole-gland extract; anterior lobe extract; thyroid gland extract.

(11) Metamorphosis is rapidly and consistently induced in the larva of *Phallusia nigra* by extracts prepared from fresh tissues of adult *Phallusia nigra*, but these *Phallusia* extracts do not induce metamorphosis in larvæ of *Polyandrocarpa tincta* and, *vice versa*, *Polyandrocarpa tincta* tissue extracts quickly induce metamorphosis in the *Polyandrocarpa tincta* larvæ but have at most a slight effect on metamorphosis of *Phallusia* larvæ.

(12) The agents extracted from whole gland preparations of vertebrate thyroid and pituitary may be endoenzymes (ferments) capable of activat-

ing reactions of a general nature occurring in the protoplasm of a wide range of animals. Hormones produced by these glands probably had nothing to do with the high percentage of metamorphosis induced.

(13) The activating agents extracted from the fresh ascidian tissues that were so specific in their effects may also be endoenzymes of a highly specialized kind, each found only in a single species of ascidian.

(14) Interpretation of the mechanism of metamorphosis as a chain of reacting substances and structures is supported by the fact that metamorphosis is induced by many diverse artificial agents. That is, any substance capable of reacting with one of the links in the chain may effectively initiate that portion of the process beginning with the link activated.

(15) The mechanism of metamorphosis is comparable in its organization to that of development of an egg, which also may be activated by numerous and diverse chemical and physical agencies.

(16) The pH of the medium, and presumably of the larval tissues, is not a determining factor in the activation or conditioning of any of the processes involved in metamorphosis of the larvæ used in this investigation.

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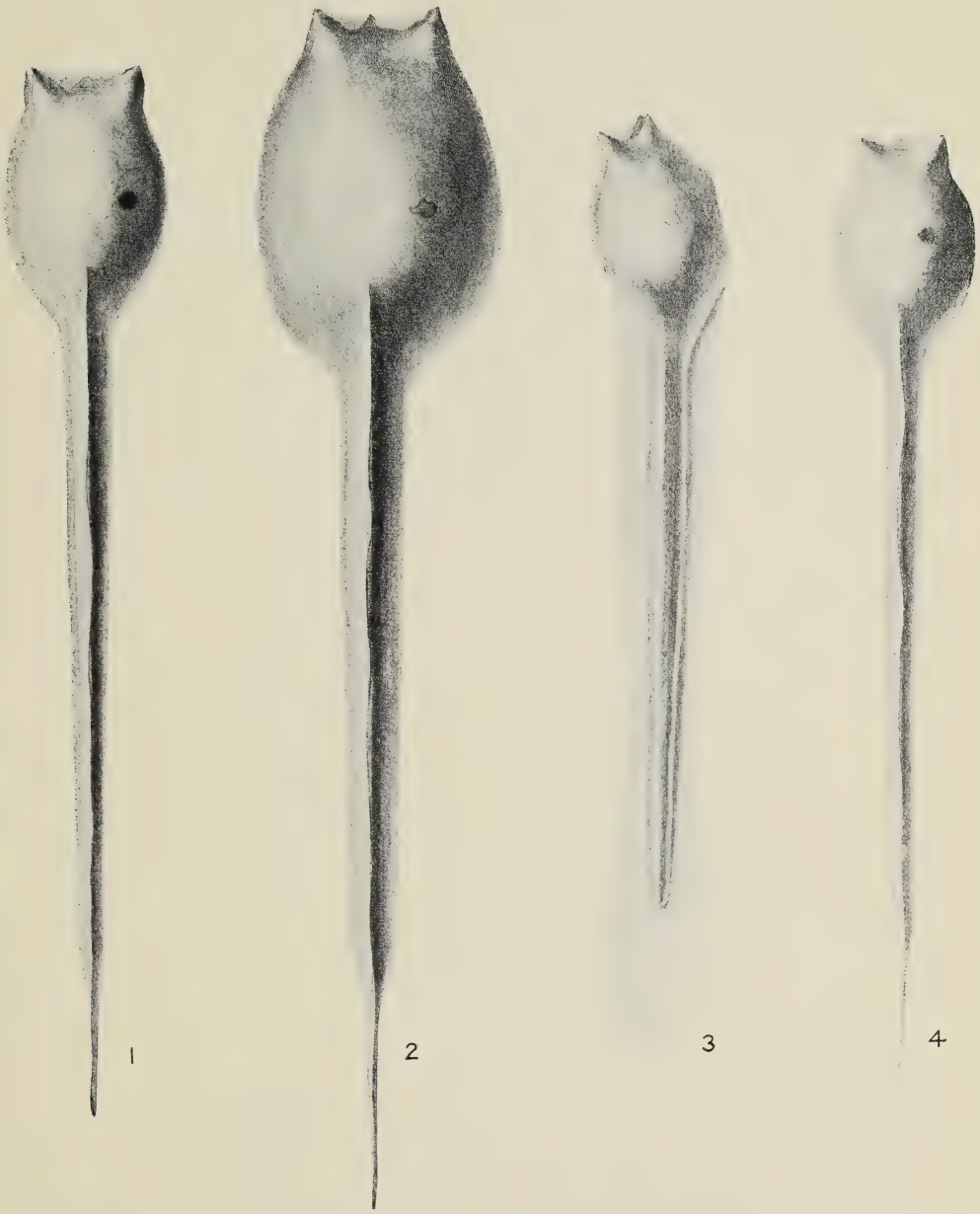
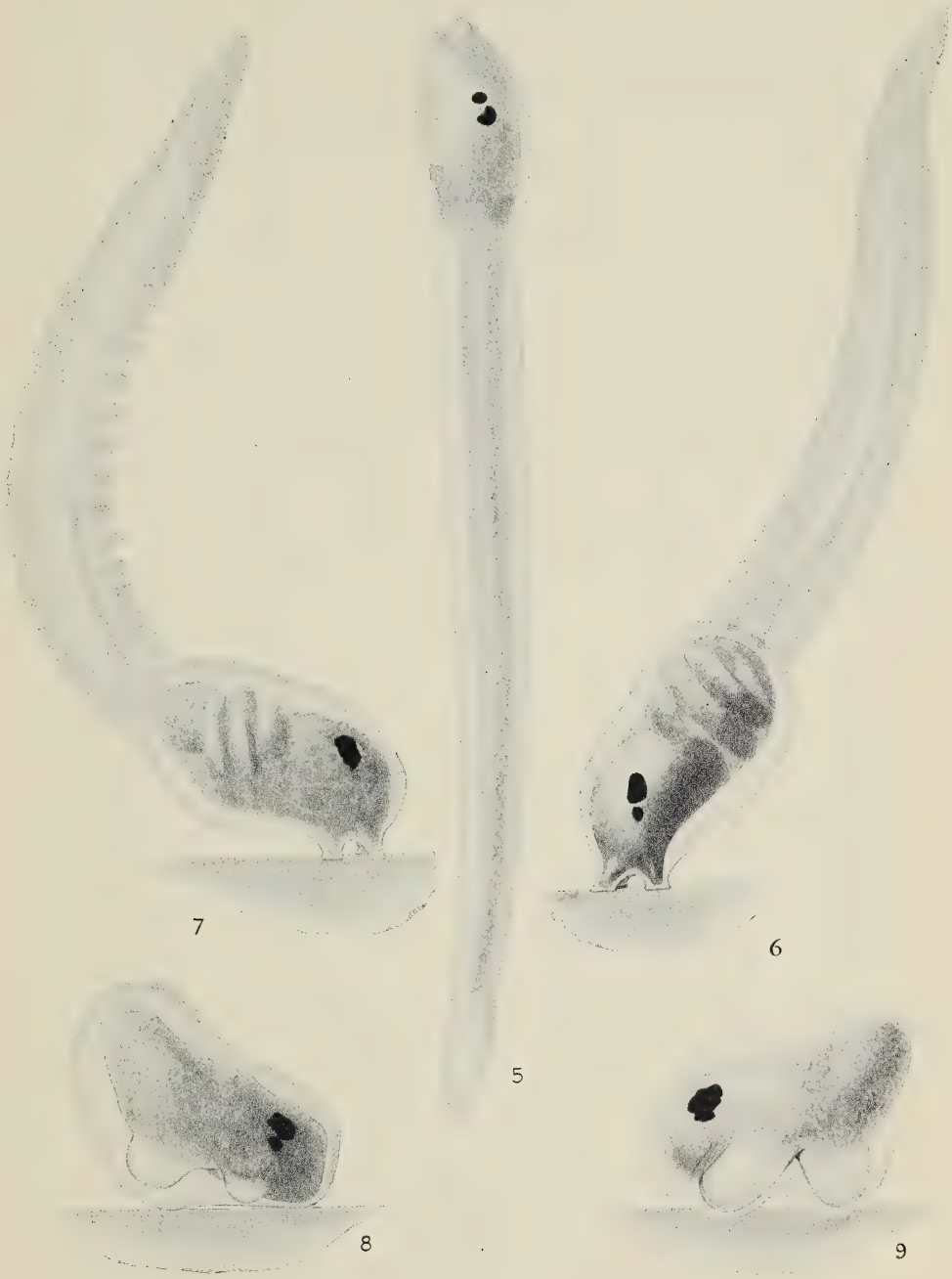


Drawings of larvæ used in the investigation of the process of metamorphosis. Fig. 1, larva of *Polyandrocarpa gravei*; fig. 2, *Symplegma viride*; figs. 3 and 4, *P. tincta*.



Drawings of the larva of *Phallusia nigra* as it appears during the free-swimming period (fig. 5), at two stages in the disruptive phase of its metamorphosis (figs. 6, and 7) and two early stages of growth of the ascidiozooid (figs. 8 and 9).

PAPERS FROM TORTUGAS LABORATORY, VOL. XXIX

XI

STUDIES ON THE CILIATES OF SEA-URCHINS

A General Survey of the Infestations Occurring in Tortugas Echinoids

By Philip B. A. Powers
Zoological Laboratory, University of Pennsylvania

With two plates and fourteen text-figures

[Issued December 9, 1935.]

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STUDIES ON THE CILIATES OF SEA-URCHINS

A General Survey of the Infestations Occurring in Tortugas Echinoids

INTRODUCTION

With the accumulation of data concerning the ciliate infestations of the alimentary tract of echinoids (Biggar and Wenrich, 1932; Powers, 1933; Yagiu, 1933 and 1934; Uyemura, 1934; Lucas, 1934) it became of increasing interest to have a complete record of the case first noted by Jacobs (1914) of the ciliates infesting sea-urchins about Tortugas. In his record of a series of physiological studies on certain protozoan parasites of *Centrechinus antillarum* (Philippi) = [*Diadema setosum* Gray], Jacobs briefly described four species of ciliates designating them respectively as forms A, B, C and D.

In order to complete the observations begun by Jacobs, and to obtain material for the descriptions and figures of these "Forms," the writer spent the summer of 1933 at the Tortugas Laboratory. During this time twelve well-defined species of ciliates were found distributed among seven species of sea-urchins. At the close of the season, a preliminary report covering the summer's results was submitted and later published in the Annual Report of the Tortugas Laboratory (Powers, 1933b).

Once the general outlines of morphology have been established for all of the members of this infestation, work may then proceed on the finer cytological details of the endoplasmic structures. The present paper, therefore, gives a complete account of the general morphology of these ciliates, as well as a description of the various associations encountered.

ACKNOWLEDGMENTS

It is with pleasure that the writer acknowledges his great debt to Doctor David H. Wenrich for his encouraging criticisms of this work during its progress. For the privileges of the Tortugas Laboratory during the summer of 1933 acknowledgments are made to the Carnegie Institution of Washington and to Doctor W. H. Longley, executive officer at Tortugas. The writer also wishes to thank Miss E. Wells of the Biological Abstracts, for advice in the proper derivation of the new specific names; as well as Doctor A. H. Clark of the United States National Museum, who established the indentity of some of the echinoids.

To Doctor Herbert F. Prytherch of the U. S. Fisheries laboratory at Beaufort, North Carolina, who made possible the collection of sea-urchins of that region; and to Doctor J. F. G. Wheeler, who forwarded fixed intestinal contents of representative Bermuda sea-urchins the writer wishes to express his appreciation.

MATERIALS AND METHODS

Although H. L. Clark (1919) lists 18 species of littorial sea-urchins common to the West Indian Region, only seven of those were obtained at Tortugas during the summer of 1933. The eighth species of sea-urchin examined was *Astropyga magnifica* A. H. Clark (1934), a new species whose nearest relative occurs on the west coast of Mexico and Central America. In table 1 are listed those species of sea-urchins whose endoparasitic infestations were investigated.

TABLE 1—*Materials (listed in order of their abundance)*

Echinoid Hosts	No. Examined	Per cent Infested	Number of Species of Ciliates Involved
Centrechinus antillarum	86	100	3 to 7
Echinometra lucunter	14	100	2 6
Tripneustes esculentus	22	100	1 5
Clypeaster rosaceus	8	100	2 6
Eucidaris tribuloides	26	...	.....
Lytechinus variegatus	14	100	2 5
Clypeaster subdepressus	3	100	2 6
Astropyga magnifica	2	100	3

In addition to these echinoids from Tortugas, the writer was able to check the intestinal protozoa of *Lytechinus variegatus* from Beaufort, North Carolina; as well as the following, whose fixed gut contents were kindly sent me by Doctor Wheeler from Bermuda:

Centrechinus antillarum (Philippi) = [*Diadema setosum* Gray]
Echinometra lucunter (Linnæus) = [*Echinometra subangularis* Desmoulius]
Lytechinus variegatus (Leske) = [*Toxopneustes variegatus* Gualteri]

In all references in this paper, the names used for the echinoids in question are those in good usage at the present time.

The routine method for examining sea-urchins and obtaining material was as follows: Freshly collected sea-urchins were rinsed in filtered freshwater and their test was opened by using a pair of heavy scissors to remove carefully the oral surface, thus preventing injury to either intestine or gonads. After draining all of the perivisceral fluid from the cœlom, the latter was rinsed with filtered sea-water in order to remove as much as possible of the perivisceral fluid which was early found to be very toxic to many of the ciliates of the alimentary tract. Then through a small puncture in the wall of one of the large pouches of the intestine, a sterile pipette was introduced to collect its fluid contents which were removed to a clean centrifuge tube and concentrated by gently centrifuging for 40 seconds at a speed of about 1080 revolutions per minute. This concentration greatly facilitated

the examination of the ciliates present in a particular host and gave an abundance of material for the preparation of smears, hanging drops, or the fixation of bulk material.

For the study of living ciliates, Grubler's Neutralrot, used in various dilutions in sea-water, was invaluable for demonstrating the contents of the food vacuoles and in many cases the ciliary pattern through coloring the basal granules.

For a study of general morphology, the most satisfactory of the fixed preparations were made by exposing ciliates to the fumes of 2 per cent OsO_4 for from 2 to 6 hours and then mounting in glycerine jelly. If the material subjected to the fumes of osmic acid be suspended on a cover-slip in a shallow drop of water and if the excess moisture be allowed to evaporate before mounting in glycerine jelly, a carefully sealed preparation will last for years. The chief merit of this method is the "wholeness of picture" it presents of an organism. Cytoplasmic structures, fat and lopoid globules, cilia and posterior bristles, delicate cytostomal structures and body configuration, all are more accurately preserved by this technique than by any other method known to the writer. Its weakness lies in the distortion which the sudden tranfer to gylcerine jelly will often bring about in very delicate and fragile organisms which have not been sufficiently hardened with the OsO_4 fumes.

For balsam mounts, material was fixed in Schaudinn's, Yocom's, or Flemming's fluid (Flemming's without acetic acid is indicated as F. W. A.); either in bulk or as smears on cover-slips. Bulk material was handled in centrifuge tubes and stored in 75 per cent alcohol in small rubber-capped ampules. Smears were made from this bulk material by the albumen method. Among the most successful stains used were: cocchineal and nigrosin, iron-alum hematoxylin, the Borrel stain and Feulgen's nuclear reaction.

CILIATES ASSOCIATED WITH TORTUGAS ECHINOIDS

In the following notes the writer has included descriptions of all of the species encountered, and even in the case of those already known additions have been made to their previous descriptions. Wherever it has been possible, the characteristics given with the discussion of each species are such as may be obtained from a study of the living organisms and whole mounts.

In dealing with flattened organisms, the side which is more often in contact with the substratum and whose surface contains the greater number of ciliary rows is designated as the ventral surface, irrespective of the position of the cytostome. Thus in Plate I all figures are drawn showing the details of the cytostome, which according to the above definition is located in the dorsal surface. Further, to avoid confusion, sides, or margins, in the sense of being to the right or left, are so designated in reference to

the oral surface, for such terms are useful in describing the extent of the peristomal structures.

All drawings for text-figures were made from fixed smear preparations, drawn with the aid of a camera lucida, at the scale indicated.

DESCRIPTIONS OF SPECIES

Genus METOPUS Claparède et Lachmann 1858

The members of this group are most conspicuous due to their size, the density of their cytoplasm and their abundance. As Kahl (1927) has noted, they constitute a very polymorphic group whose more exaggerated types are indeed bizarre. These deformities are usually associated with an abnormal development of the frontal area (crest) and peristomal membranelles, and are found to effect about 0.1 per cent of a population.

Metopus circumlabens Biggar and Wenrich

(Plate I, fig. 1; text-fig. 1.)

Diagnosis—Average length 100 μ, width about 0.5 length. Body flat, shape obovate, posterior and elongated, bearing tuft of cilia, extensive frontal region bearing band of cilia. Well-marked peristome containing strong membranelles sweeps over antero-dorsal surface, distal end bending posteriorly with well-defined undulating membrane in cytopharynx. Pharyngeal strand extensive. Numerous contractile vacuoles. Macronucleus irregular. Micronucleus single.

Description—Average length 100 (70 to 165) μ; average width 60 (50 to 75) μ. Body is flattened, being about 20 μ thick anteriorly, increasing in thickness posterior to the oral groove. The anterior region or crest is rounded and overhangs the oral groove. Posteriorly the body tapers gradually, ending in a blunt cone. The ventral surface is slightly concave; its 21 striations arise in an area surrounding the left margin of the oral groove and curve posteriorily, following the general configuration of the right margin. Dorsad (posterior to the oral groove) the body bulges with a marked convexity. The 13 widely spaced dorsal striations arise near the lower margin of the peristome and extend caudad, running parallel to the longitudinal axis.

As in other members of this genus, the ingestatory apparatus is highly differentiated and consists of an external peristome or oral groove, and a cytopharynx, all associated with the anterior and right margins of the dorsal surface. Each of these divisions has special structures. The external oral groove and cytopharynx have along their floor a row of about 60 strong membranelles. In addition, the cytopharynx contains, in a recess along its right margin, a well-developed undulating membrane. Lucas (pl. IV, fig. 17, 1934) figures a component cilium of this undulating membrane coming out of the right margin of the peristome. Associated with the cytopharynx is an extensive pharyngeal strand. This strand appears to be associated with the fibrillar system and may be followed posteriorly into the deep endoplasmic region where it terminates as a fine coil.

The cilia are more or less uniform in length over the surface of the body, measuring from 6 to 12 μ. Beginning near the anterior limit of the undulat-

ing membrane, a band of 5 rows of closely placed cilia curves along the crest above and parallel to the peristome. Other than this band, the crest is covered with a smooth, bare, anterior field. The posterior end is conspicuous because of a trailing tuft or brush of from 15 to 25 cilia, the longest of which are from 25 to 40 μ long.

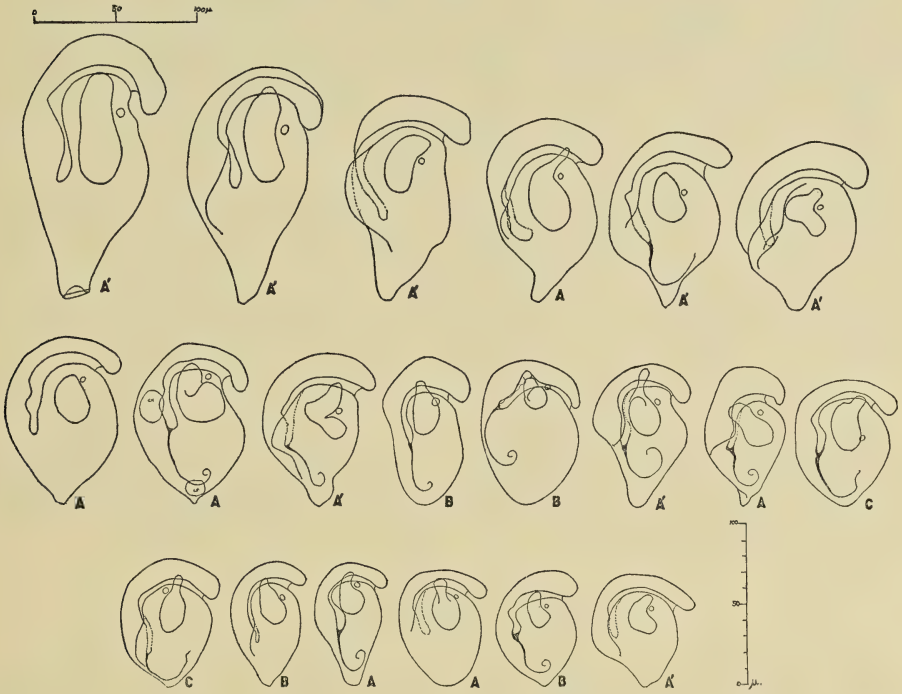

FIG. 1—*Metopus circumlabens* Biggar and Wenrich. Specimens represent a composite population formed from representative samples showing range in size and shape, and configuration of the macronucleus. Individuals A are from *Centrechinus antillarum* of Bermuda; others are from Tortugas hosts: A^1 from *C. antillarum*; B from *Tripneustes esculentus*; and C from *Echinometra lucunter*. All drawings are from camera lucida outlines of individuals fixed in Bouin's or Schaudinn's.

Because of the converging striations, the cilia near the left border of the oral groove appear to form a brush when viewed from the dorsal surface.

The endoplasm is dense, granular and filled with food vacuoles in the region posterior to the macronucleus. The food vacuoles seem filled with bits of algæ and bacteria. A definite cytopyge is located in the tip of the posterior end. Since this species will readily ingest lampblack, the course of the food vacuoles through the cytoplasm can be easily established. The contractile vacuoles are of two types: One type, found near the right and left margins, seems to be for the purpose of collecting liquid, for as soon as either of these lateral vacuoles is filled a small passage-way leading to the posterior contractile vacuole appears in the endoplasm and the content of the collecting vacuole is partially discharged. The filled posterior contractile vacuole then empties itself to the exterior through a small excretory pore near the cytopyge.

The macronucleus is usually irregular in shape (see text-fig. 1), its anterior portion sometimes extending back of the oral groove and up into the crest. A single micronucleus is present, usually located near the left margin of the macronucleus. Fission has been observed, but conjugation has not been noted.

Hosts—Usually abundant in *Centrechinus antillarum*, not so plentiful in *Echinometra lucunter*, and occasionally found in *Tripneustes esculentus*.

Remarks—Through the kindness of J. F. G. Wheeler, collections of material from the intestine of two specimens of *Centrechinus antillarum* taken at Bermuda were available for making comparative studies of *Metopus circumlabens* of the region from which it was first described with those from Tortugas. The general result from a study of all available groups of *M. circumlabens* was a difference in the range of size, whose extremes were found to vary slightly among the groups taken from different specimens of the same host, as well as among groups from different "specific" hosts. Thus a combined population (text-fig. 1), with samples from the extremes and means of the four groups studied, shows that the greatest range in size occurred among specimens in *C. antillarum* from Tortugas, and that the members of the other groups are somewhat smaller.

The distribution of *M. circumlabens* seems confined to sea-urchins living within a depth of 3 fathoms, for in none of the possible hosts taken from deeper parts of the littoral zone was it ever found. It was abundant in *Centrechinus antillarum*, taken from about 2 fathoms. In one *Tripneustes esculentus*, taken just below water on the reef, was found the densest infestation ever noted, in spite of the fact that this host in general is rarely infested with *Metopus*.

Metopus rotundus Lucas

(Plate I, fig. 4; text-fig. 2)

Diagnosis—Average length 180μ, width about 0.7 of length. Body flat, shape circular to oval; posterior end slightly tapered or round, bearing tuft of long cilia. Ciliary band extends along crest parallel to external oral groove. Cytopharynx almost as long as peristome. Undulating membrane and pharyngeal strand small in proportion to cytosome. Numerous contractile vacuoles. Macronucleus spherical, micronucleus single.

Description—Average length 180 (110 to 300)μ, average width 115 (90 to 200)μ. This species displays the same basic morphological characters as are present in *M. circumlabens*, its differences being in the degree of their development. One distinguishes *M. rotundus* on the basis of its size and shape and the general opacity of the body due to the density and thickness of its endoplasm.

The oral apparatus, while in reality larger and more stoutly constructed, is proportionately smaller than that of *Metropus circumlabens*, the crest and peristome both being relatively smaller. The cytopharynx extends deeply into the endoplasm; with an undulating membrane near the base, fitted in a shallow recess in the right wall. The pharyngeal strand is rather short, diminishing in size to the distal coil. Prominent and correspondingly heavier membranelles cover the floor of the oral groove.

The striations are very close together, there being about 36 on the dorsal surface and about 50 on the ventral surface. The body cilia are about 12μ long and closely set, those in the posterior end measuring from 25 to 60μ.

In the living specimens, the configuration of the posterior end varies from almost round to a slightly tapering cone, a condition due possibly to the volume of the endoplasm. In most of the fixed specimens, the posterior end is rounded in a state of contraction caused by fixation.

Two kinds of endoplasm may be differentiated; one, a mass of lightly granular homogeneous material, filling the area below the oral groove and surrounding the macronucleus; the other is coarser, made up of a mixture of lipoid globules, metaplastids and vacuoles. Contractile vacuoles may be either collecting or excretory; the collecting vacuoles are usually found

FIG. 2—*Metopus rotundus* Lucas. Showing variability in size, shape of body outline and of nuclei.

in the mid-region near the margin, while the excretory vacuole is in the posterior region. Several small accessory vacuoles are found about the posterior contractile vacuole. The food vacuoles are many and of various sizes according to their contents; some are filled with bacteria, others with algæ or sperm cells (from contamination with gonad during removal from sea-urchin), and occasionally an ungulfed *Anophrys elongata* will be observed, recognized because of its glycogen bodies. The cytopyge is located in the posterior end.

A single micronucleus is found usually near the anterior margin of the spherical to ovoid macronucleus. Individuals in fission or conjugation were not observed.

Hosts—Centrechinus antillarum is the only sea-urchin from which this species has been recorded. In the Tortugas region, it was present in about 12 per cent of the animals examined and never in abundance. From 5 to 15 could be obtained for every 100 individuals of *M. circumlabens*.

Remarks—M. rotundus moves slowly and may be collected with aid of a fine pipette. Specimens will often fasten themselves to the substratum so firmly that considerable effort must be made to dislodge them.

In this form, as in *M. circumlabens*, the variation in size is considerable (see text-fig. 2), and while there are few individuals near the 300μ limit, they are seen upon rare occasion.

M. S. Lucas (1934) reports this form as the sole infestant of three specimens of *Centrechinus antillarum* from Bermuda. At Tortugas *M. rotundus* was always found in company with other ciliates (table 2).

Metopus brevicristatus n. sp.

(Plate I, fig. 3; text-fig. 3)

Diagnosis—Average length 50μ, width about 0.6 of length. Body flat, shape ovoid, posterior slightly pointed, bearing a tuft of long cilia. Transverse peristome near anterior end, reducing extent of crest. Free end of crest is blunt. Irregular macronucleus accompanied by a single micronucleus. Contractile vacuoles numerous.

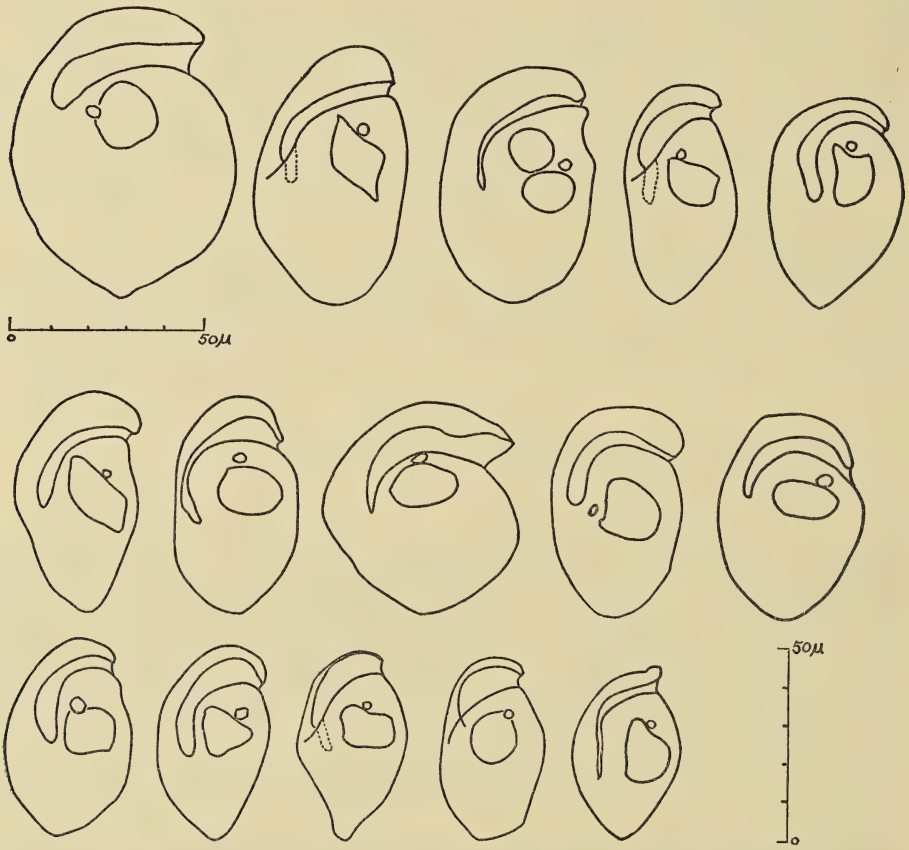

FIG. 3—*Metopus brevicristatus* n. sp. Showing range of variability for size, shape of body outline and of nuclei.

Description—Average length 50 (40 to 80)μ, average width 30 (20 to 45)μ. This is the smallest species of *Metopus* found in association with the Tortugas echinoids. Other than size, *M. brevicristatus* is characterized by the smallness of its frontal crest and the irregularity of its macronucleus. The cytopharynx, accompanied by a short undulating membrane, extends into the median cytoplasm for about one-fourth the length of the oral groove. The pharyngeal strand sends its fibers posteriorly, ending as a fine

terminal coil. The endoplasm is light, almost colorless. Food vacuoles are filled with bacteria. The ciliary band is closely applied to the anterior peristomal margin, its long cilia covering the barren dorsal surface of the slight anterior field.

Host—Abundant in *Clypeaster rosaceus* and apparently limited by a definite specificity to that host.

Remarks—While the upper limits of *M. brevicristatus* (text-fig. 3) overlap the lower limits for the size variability of *M. circumlabens*, it forms nevertheless a homogenous group, limited to a specific host and clearly defined by its dwarfed anterior crest.

Metopus histophagus n. sp.

(Plate I, fig. 2; text-fig. 4)

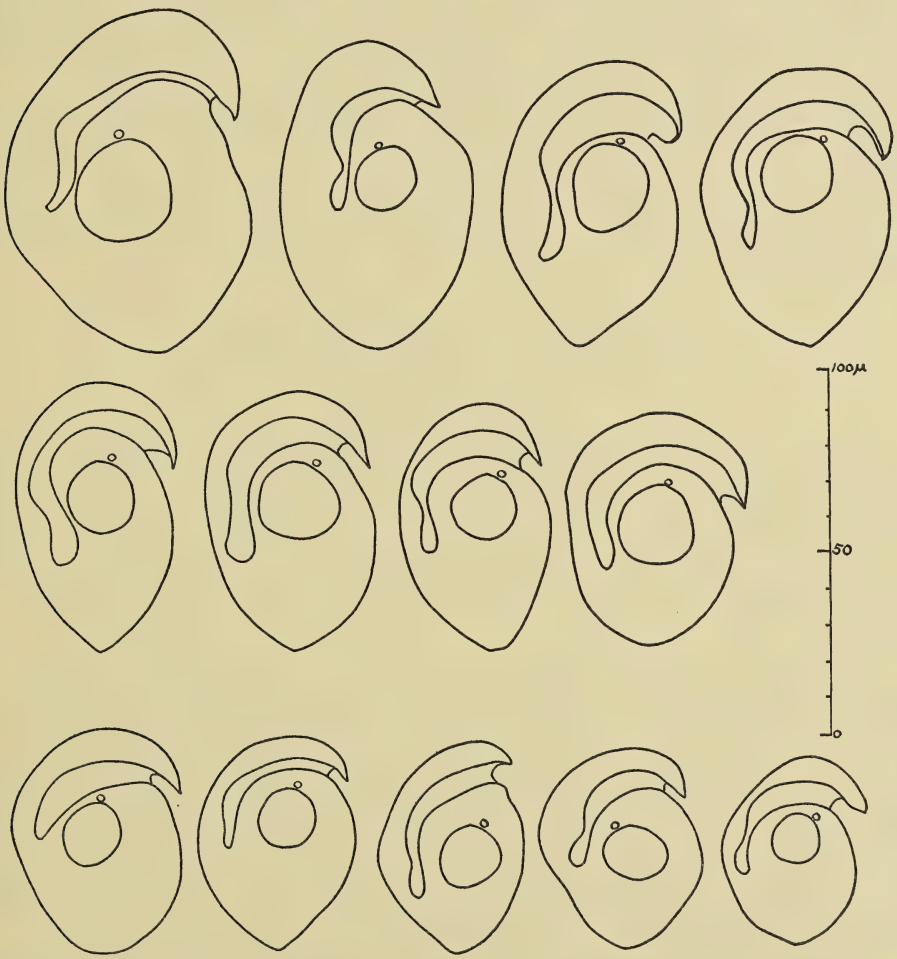

FIG. 4—*Metopus histophagus* n. sp. Showing range of variability for size, shape of body outline and of nuclei.

Diagnosis—Average length 70μ, width about 0.7 of length. Body flat, shape ovoid, posterior slightly conical bearing tuft of long cilia. Cyto-

pharynx extends well into median endoplasm. Free end of crest sharply pointed. Single micronucleus usually anterior to spherical macronucleus. Food vacuoles contain epithelial cells from intestinal mucosa of host.

Description—Average length 70 (50 to 90)μ, average width 45 (35 to 70) μ. In size, *M. histophagus* falls between *M. circumlabens* and *M. brevicristatus*. The frontal crest is well developed and comes to a pronounced point on the left margin, overhanging the origin of the peristome. The 5 rows of long cilia which form the crestal band, closely parallel the peristome. A cytopharynx, containing a well-defined undulating membrane, encloses about one-third of the oral groove. The pharyngeal strand arises from the delicate terminal coil in the posterior endoplasm, to form a pronounced fibrillar structure surrounding the base of the cytopharynx. The food vacuoles filling the posterior endoplasm contain distinct epithelial cells whose nuclei appear to be identical with those of the intestinal mucosa of the host; hence the specific name, *histophagus* = (tissue feeder). Numerous simple contractile vacuoles are present. The macronucleus is usually spherical and is accompanied by a single anterior micronucleus.

Host—M. histophagus was observed only from *Clypeaster subdepressus* where it was found in abundance among the débris of the alimentary canal. Unfortunately the season's collecting brought in only a few specimens of *C. subdepressus*, and while all of these specimens were infested their number was not sufficient to allow an exhaustive survey of this parasitic ciliate in its living state.

Genus CRYPTOCHILIDIUM Schouteden 1906

Cryptochilidium bermudense (Biggar and Wenrich)

(Plate 2, fig. 4; text-fig. 5)

Diagnosis—Average length, 155μ, width about 0.43 of length. Body broad and flat, leaf-like anterior end with row of pronounced lappets along aboral margin. Oral margin parallel to antero-posterior axis with cytostome located posterior to median line. Posterior end bulbous, terminating in caudal projection bearing numerous long bristles. Cytostome contains two thin membranes as well as a field of buccal cilia. Food vacuoles are limited to area adjacent to cytostome. Macronucleus spherical, accompanied by single micronucleus.

Description—Average length 155 (54 to 210)μ, average width 65 (40 to 98)μ. First observed at Tortugas in 1910, this species was designated by Jacobs as form "D." Its first description was published by Biggars and Wenrich in 1932, from material collected at Bermuda. Since that time the writer has examined material collected at Beaufort, North Carolina (Powers 1933a, 109). It was therefore with interest that this species was studied at Tortugas, the locality of its original discovery. Other than enlarge the range of variability and confirm several observations on the structure of the lappets and cytostome, the writer is not able to enrich the descriptions of the general morphology as previously published.

Structurally, the lappets appear to be projections of the smooth pellicle of the anterior field bordering the aboral margin of the ventral surface. The number of these projections may vary from 18 to 28. While the drawings of Mrs. Biggar which were made from life indicate that cilia are inserted in these lappets in tufts and bunches, in material well fixed and stained the

pattern of the basal granules indicates that these anterior cilia arise from the striations of the dorsal surface.

The delicate cytostomal structures are somewhat obscured because of the dense long cilia which overhang the cytostome and fill the buccal cavity. In notes made at the time of observation, there is recorded the single instance where the cytostomal membranes were observed to be moving vigorously, as were also the cilia of the buccal cavity. Whether the movement of these membranes was independent of, or concurrent with, the movement

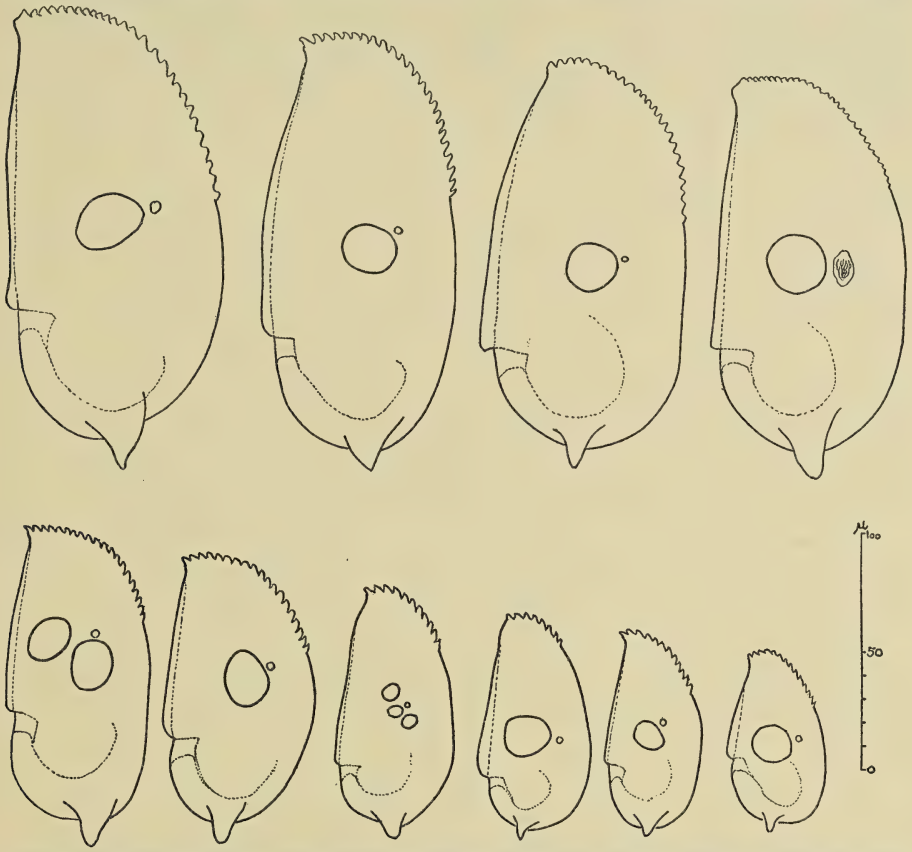

FIG. 5—*Cryptochilidium bermudense* (Biggar and Wenrich). Showing range of variability for size, shape of body outline and nuclei.

of these buccal cilia could not be determined, for the writer was never again able to observe movement in this region.

Hosts—This ciliate, though delicate and susceptible to changes in environment at the hand of the observer, was found present in all of the species of echinoids in which infestations were noted. In general it appeared as the predominating form in the echinoids dredged from the lower range of the littoral zone (30 to 50 fathoms).

Remarks—Often the gut contents of *Lytechinus varigatus* would be swarming with individuals, many of which would be in fission. Conjugation was not observed, although many individuals showed evident signs of macronuclear reorganization.

Genus ANOPHRYS Cohn 1866

Anophrys elongata Biggar and Wenrich

(Plate 2, fig. 1; text-fig. 6)

Diagnosis—Average length 96μ, width from 0.2 to 0.3 of length. Body elongated; flattened to circular in cross-section. Lateral cytostome located at base of a thin mobile probosis which extends anteriorly for 0.2 to 0.3 of total length. A row of long cilia extends from the aboral margin of cytostome to the anterior end. Endoplasm usually filled with glycogen granules of varying size. Central and spherical macronucleus accompanied by a single micronucleus. Posterior region contains large contractile vacuole and bears long, fine terminal bristle.

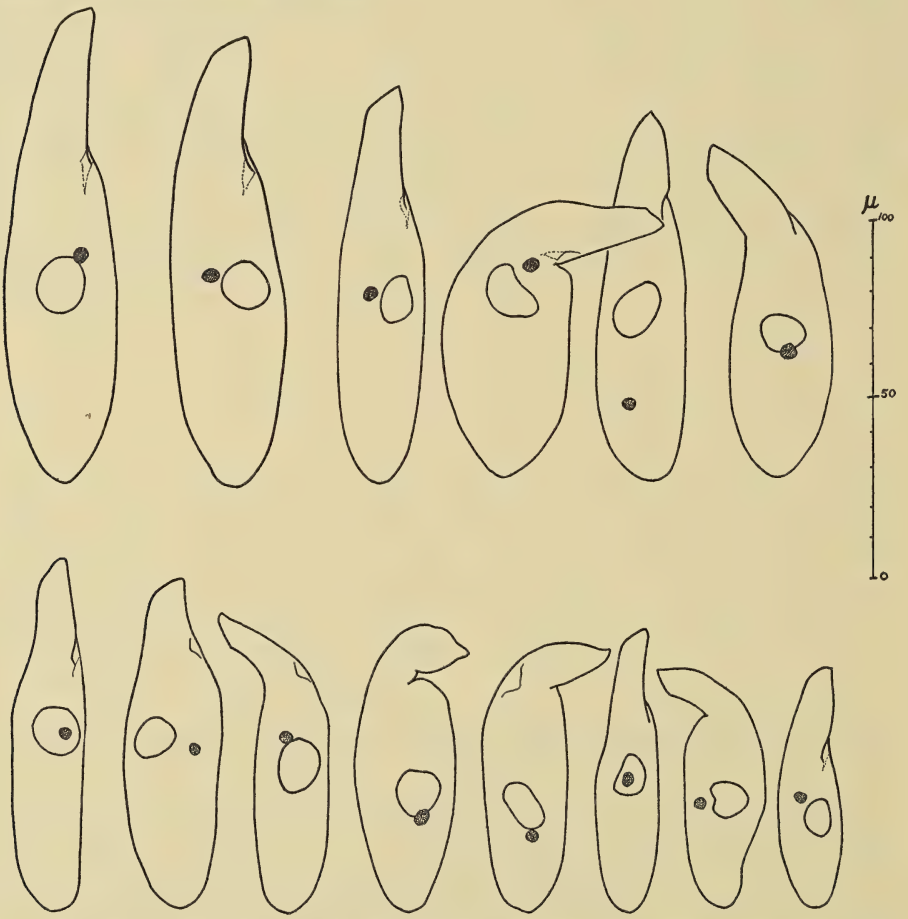

FIG. 6—*Anophrys elongata* Biggar and Wenrich. Showing range of variability for size, shape of body and nuclei.

Description—Average length 96 (67 to 128)μ; average width 22 (16 to 33)μ. In shape the body is almost linear, with the anterior region thinning out to a point. This anterior region is very labile and can be moved about, bending this way or that.

The cytostome is small, obscure, and in the best preparations appears to open into a small vestibule. The internal pellicle of the vestibule protrudes externally as a pair of thin membranous flaps. These flaps were never seen in motion. From the vestibule, a short cytopharynx leads into the interior endoplasm. Bordering the aboral margin of the cytostome and extending anteriorly is a row of long cilia. These cilia are closely placed and vary in length from 12 to 16μ. They were never observed to simulate a membrane.

Food vacuoles could not be observed in the process of formation; however when present they seemed to be filled with micrococci. If bits of intestinal mucosa from the host were placed on a slide with a drop of seawater containing *A. elongata,* the latter would gather about the fragment of tissue apparently feeding; likewise this species could be seen cutting through the bodies of dead *Metopus,* rupturing vacuoles and seeming to feed on the remains.

Most characteristic of this species are the large quantities of glycogen granules which may be stored in the endoplasm. These granules vary in length from 2 to 4μ and are about 2μ in diameter. In a few individuals, glycogen granules of much greater size were found. This animal starch is usually confined to the endoplasm posterior to the cytostome and often this region will be so filled with starch granules that the animal's body will be greatly swollen and distorted.

The body cilia are about 6μ long and are arranged in striations running parallel to the body axis. Due to the narrowness of the anterior end and the convergence there of the ciliary rows, the cilia here are very numerous. A slight anterior field runs along the anterior margin. The terminal cilium measures from 15 to 22μ long and is very fine, arising from the middle of a bluntly pointed posterior end.

Hosts—All sea-urchins capable of an infestation were found to be possible hosts of this form. Those sea-urchins dredged from 30 to 50 fathoms were always heavily infested with *A. elongata.*

Remarks—Compared to specimens from Bermuda, there are no detectable variations in size, shape or internal characteristics. Neither fission nor conjugation were observed, although many individuals from different hosts were examined.

Anophrys aglycus n. sp.

(Plate 2, fig. 2; text-fig. 7)

Diagnosis—Average length 81μ, width about 0.25 of length. Body flattened, slightly sigmoid in shape of curvature; posterior end pointed, bearing long bristle. Cytostome a long narrow opening in anterior 0.2 of body. A row of very long peristomal cilia form an undulating membrane along inner margin of peristome. Endoplasm is colorless and lacks glycogen; food vacuoles numerous and filled with micrococci. Contractile vacuole located in posterior region. Macronucleus spherical located in mid-body region.

Description—Average length 81 (56 to 120)μ; average width 23 (16 to 35)μ. The shape of this species is characterized by the reverse flexure of its lateral outlines, giving it a slight sigmoid characteristic. This body shape is maintained during locomotion and feeding. Viewed sidewise, the body is very thin, being from 8 to 14μ in thickness. The body cilia measure about 16μ in length and are arranged in rows which run parallel to the longitudinal axis. The terminal bristle measures from 16 to 20μ long, a little heavier than the body cilia, apparently somewhat of a tactile spine. The

peristome is a long oblong slit in the anterior portion of the body. Along its internal margin a row of long cilia are prominent and in life simulate an undulating membrane. The cilia composing this peristomal membrane are no longer than the body cilia; however, they are much finer and are more closely set. Thus the body cilia are about 2μ apart, while these peristomal cilia average about three per micron.

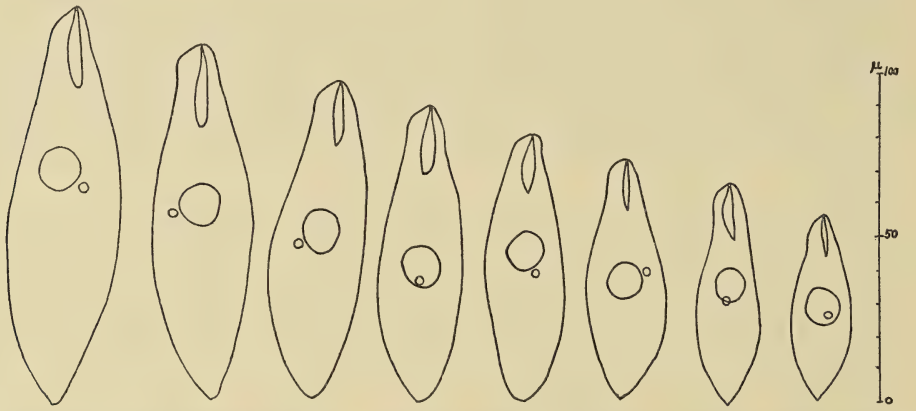

FIG. 7—*Anophrys aglycus* n. sp. Showing range of variability for size, shape of body outline and nuclei. Sketches were taken OsO_4 preparation.

Other than the position of the mouth and the general body contour, *A. aglycus* is further distinguished from *A. elongata* by the absence of glycogen granules in its endoplasm. Repeated tests with iodine vapor on numerous random samples of specimens have failed to reveal any substance in the endoplasm corresponding to animal starch. While colorless, the endoplasm contains numerous fat droplets and many food vacuoles filled with micrococci. The spherical macronucleus measures about 10 to 12μ in diameter, is accompanied by a single micronucleus and occupies a position slightly anterior to the middle of the body. Fission was observed upon two occasions. The large contractile vacuole, located in the posterior region was never observed in systole and hence its behavior is thought to be very slow. Locomotion as observed under a cover-slip was very erratic, lacking in uniformity of course or speed.

Hosts—While seldom present in any great numbers, *A. aglycus* is common to all sea-urchins living near the tide line. *Tripneustes esculentus* collected on the reef offered the best examples of infestation with this form.

Remarks—With first glance at a mixed infestation, it is difficult to separate these two superficially similar species of *Anophrys*, for their differences in size and shape are not immediately apparent among a large mixed group of animals in motion. Moreover, *A. aglycus* is seldom present in great numbers.

Genus COHNILEMBUS Kahl, 1933

Kahl has adopted the name *Cohnilembus* in preference to *Lembus* Cohn 1866, as the latter is preoccupied by *Lembus* A. C. Günther (Cat. Fish. Brit. Mus., vol. 2, 501 and 505, 1860. Pisc. Teleost).

Cohnilembus cæci n. sp.

(Plate 2, fig. 3; text-figs. 8 and 9)

Diagnosis—Average length 60μ, width about 0.2 of length. Body elongate and cylindrical. Anterior end pointed, posterior end bulges slightly and bears a long terminal cilium. Body cilia arranged in 10 longitudinal rows. Peristomal furrow extends length of anterior third of body and carries two undulating membranes along right border. Cytostome opens into vestibule from base of which cytopharynx leads into median endoplasm. Spherical macronucleus, usually central in location, accompanied by single micronucleus. Food vacuoles filled with bacteria. Simple contractile vacuole located posteriorly.

Description—Average length 60 (32 to 92)μ. Body is long and slender, graceful in movement. The ciliary rows are 10 in number and run parallel to the antero-posterior axis, except in the region of the peristome, where they are deflected slightly to one side in order to pass about the cytostome without interruption. The body cilia are about 7μ long and are not thickly set, being about 1μ apart. The caudal cilium is about 17μ long.

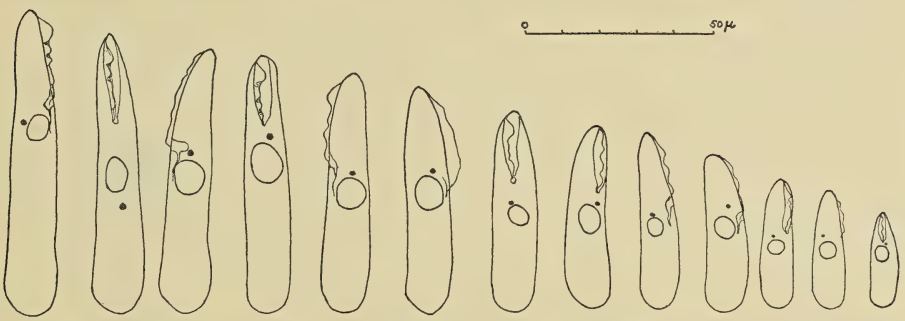

FIG. 8—*Cohnilembus cæci* n. sp. Showing range of variability for size, shape of body outline and of nuclei. In most cases only one peristomal membrane is figured.

The cytostome is located slightly above the middle of the body, at the base of a long peristomal furrow. This furrow is very shallow, about 5 to 8μ wide and carries along the length of its right margin a pair of undulating membranes. Normally the peristome extends anteriorly one-third or more the length of the body; however with the death of the individual, this distance is lessened, due to a slight contraction of the anterior region. The exact number of undulating membranes could not be determined with certainty from a study of living material, though preparations of smears and sections demonstrate clearly that there is an external as well as an internal membrane; both of which extend along the right margin of the peristomal groove. The outer and inner membrane lie very close together but are attached at their base, one to either side of the cytostome. It is possible that they are used in sweeping food (bacteria) into the buccal cavity. The cilia composing these membranes are very fine and their individual presence is seldom indicated.

Unlike *Cohnilembus pusillus* in which food goes directly from the buccal cavity into a primary food vacuole, *C. cæci* possesses a well-defined cytopharynx which extends from the floor of the buccal cavity into the interior median endoplasm following a well-defined pharyngeal fiber. Food vacuoles

which fill the endoplasm posterior to the macronucleus are filled with bacteria. The presence of these bacteria could be demonstrated either through the use of neutral red or from sectioned material. Characteristic of this

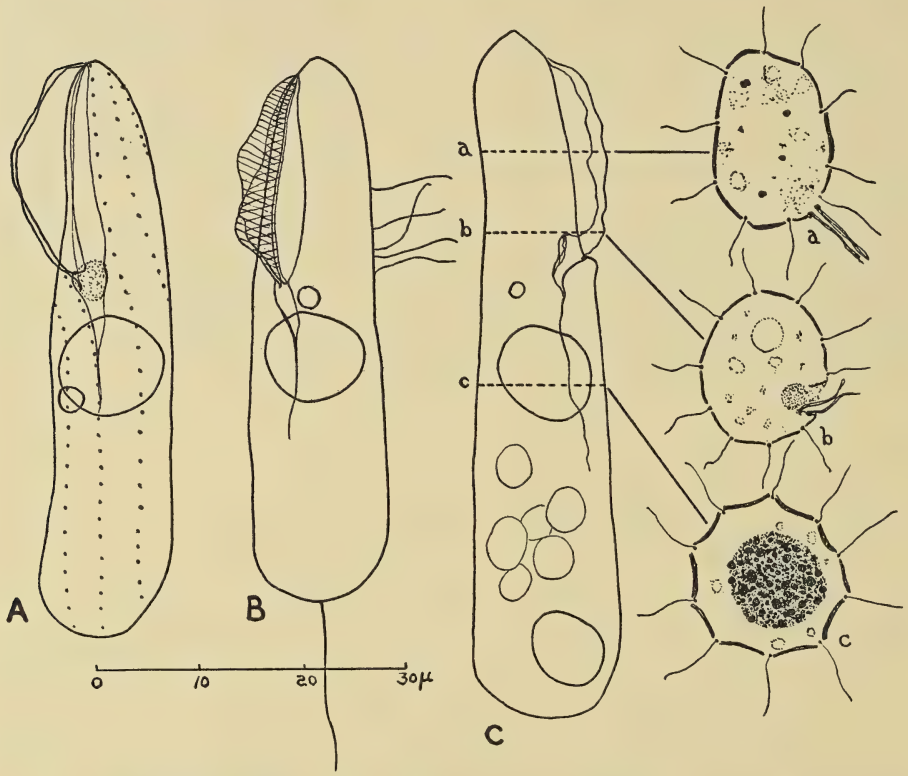

FIG. 9—*Cohnilembus cœci* n. sp. A, showing ciliary pattern of striations about the peristome. B and C, showing peristomal membranes and a trace of the pharyngeal fiber; a, b and c, represent cross-sections through respective regions of C.

species are groupings of lipoid granules which may be located anteriorly near the anterior field, posteriorly about the contractile vacuole, or grouped about the cytostome.

Hosts—This species is commonly found in any of the littoral echinoids. It has, however, a marked predilection for *Tripneustes esculentus* and in many specimens of the latter it forms the greater part if not the entirety of the infestation.

Remarks—Because of its great variability in size, the chief criterion for the identification of *C. cœci* has been its general configuration and the number of striations.

In examining some material taken on August 30, 1933, from *Lytechinus variegatus* at Beaufort, North Carolina, the writer found numerous specimens of *C. cœca.* These had not been noticed in the Beaufort sea-urchins before, due to their close resemblance to *Anophrys vermiformis,* and it was only because the attempt to identify the Tortugas *C. cœci* that they were discovered among the Beaufort group.

Compared to *Anophrys echini* Di Mauro (1904), from *Strongylocentrotus lividus* of the Mediterranean, the body size and number of striations agree so favorably as to make the two forms synonymous. However, *Anophrys echini*, from the material in our collection, possesses a cytopharynx which is much more pronounced than that of *Cohnilembus cœci*; and from a study of whole mounts only, the number of striations appear to be 6 or 8 (*C. cœci* has 10). Moreover, since this form was originally described as having only one peristomal membrane, and since the specimens at hand are not sufficiently perfect to ascertain definitely the actual number of membranes, the writer does not feel justified at this time in doing more than point out the very close relationship of these two forms.

Genus CYCLIDIUM O. F. Muller 1786

Cyclidium rhabdotectum n. sp.

(Plate 2, fig. 5; text-figs. 10 and 11)

Diagnosis—Average length 25μ, width about 0.6 of length. Body almond-shaped. Peristome a shallow groove extending about one-third length from anterior end. Two groups of cilia are associated with the peristome: one group hangs down furrow, being inserted just below anterior field; the other forms a row bordering the right margin. Surface pellicle covered with bacteria placed between ciliary rows. Posteriorly, body tapers to blunt point and bears long cilium. Spherical macronucleus accompanied by single micronucleus. Anterior field well marked and protrudes as a rounded area covered with thickened pellicle.

Description—Average length 28 (20 to 40)μ; average width 16 (12 to 22)μ. The almond-shaped body is flattened and from 8 to 10μ thick. The anterior end is well marked with a prominent anterior field, about the base of which arise the ciliary rows. The body cilia are about 10μ long and arranged in about 10 longitudinal striations. The surface pellicle between the ciliary rows is covered with bacteria. These bacteria are rods about 1μ in diameter and from 2 to 3μ in length. Within 50 seconds of death, all of these rod-like bodies loosen their hold on the organism and move away, leaving behind them an impression of their form and position on the surface pellicle.

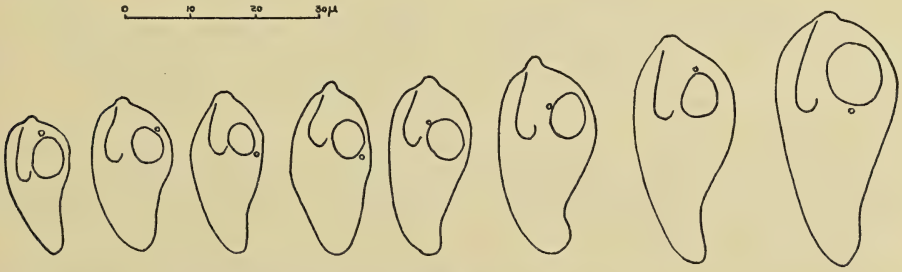

FIG. 10—*Cyclidium rhabdotectum* n. sp. Showing range of variability for size, shape of body outline and of nuclei.

The peristomal furrow arises from the cytostome and extends to the anterior field, a distance of about one-third the length of the body. Associated with the peristome are two groups of cilia; a row of long cilia border-

ing the right margin of the peristome and a tuft of cilia inserted just beneath the anterior field and hanging down the peristomal groove. The cilia along the right peristomal margin did not appear to circumscribe the posterior border of the peristome; neither did they form an undulating membrane. As for the cilia found in the anterior region of the peristome, there is some doubt as to their origin. The general impression gained from a study of whole mounts is that of a short row of long cilia coming out of the anterior region of the left peristomal margin. This point is still obscure and must be further examined in sectioned material.

The thickness of the body is greater to the right of the peristome, so that the latter lies in a cleft a little to one side of the median axis giving the shape a slight asymmetry. The posterior end is blunt and bears a long delicate cilium.

The macronucleus is ovoid to spherical in shape and is usually anterior in position, accompanied by a single micronucleus. Numerous examples of fission were noted; conjugation, however, was not observed. During fission, the bacteria persist, completely covering the free surface of the animal.

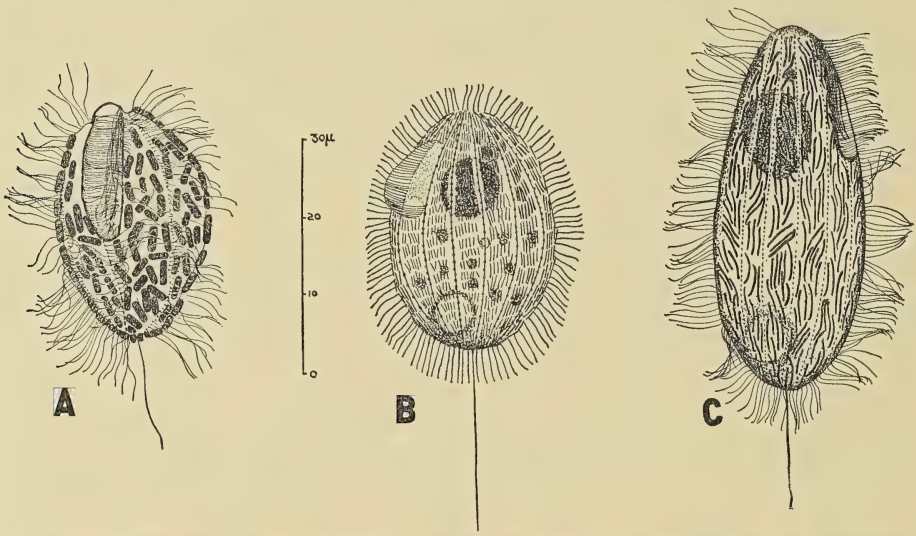

Fig. 11—Three species of *Cyclidium* known to inhabit the intestine of sea-urchins. A, *C. rhabdotectum* n. sp. found in Tortugas echinoids; B, *C. ozakii* Yagiu from *Anthocidaris crassispina* on the Pacific coast of Japan, after Yagiu; C, *C. stercoris* nom. nov., from *Strongylocentrotus dröbachiensis* of the Bay of Fundy. All three species show characteristic rod-shaped bacteria adhering to the pellicle between ciliary rows.

The posterior region of the interior endoplasm was usually filled with food vacuoles whose contents consisted wholly of bacteria. There were no indications of a cytopharynx, food passing directly from the cytostome into the primary food vacuole.

Hosts—*Cyclidium rhabdotectum* was found abundantly in about 10 per cent of the specimens of *Centrechinus antillarum* and *Echinometra lucunter* examined. When present, this ciliate usually occurred in great numbers.

Remarks—This species is exceedingly sensitive to any changes in its external environment; and while its life is usually a matter of a few minutes under a cover-slip, in hanging-drop preparations it may live several hours. This form is quite likely the species which Jacobs described as form "A."

The genus *Cyclidium* appears to be represented in two other localities in a similar rôle. From *Strongylocentrotus dröbachiensis* in the Bay of Fundy a small ciliate, whose pellicle was covered with bacteria, was found and described (Powers, 1933) temporarily under the name of *Uronema sociale;* likewise Yagiu (1933) described from the intestine of *Anthocidaris crassispina* from the Sea of Japan, *Cyclidium ozakii,* whose body was also covered with bacteria. These three species are shown for comparison in text-figure 11, all drawn to the same scale.

Cyclidium stercoris nom. nov. for Uronema sociale Powers 1933

Because *Uronema sociale* Powers 1933 is a homonym of *U. sociale* Penard 1922, the name *Cyclidium stercoris* is recommended in place of the homonym, which under article 36 of the International Rules of Nomenclature must be suppressed. This form has been removed from the genus *Uronema,* because it was found to possess a series of anterior peristomal cilia which point to its close relationship with *Cyclidium.*

Genus ENTODISCUS Madsen 1931

Entodiscus sabulonis n. sp.

(Plate 2, fig. 6; text-fig. 12)

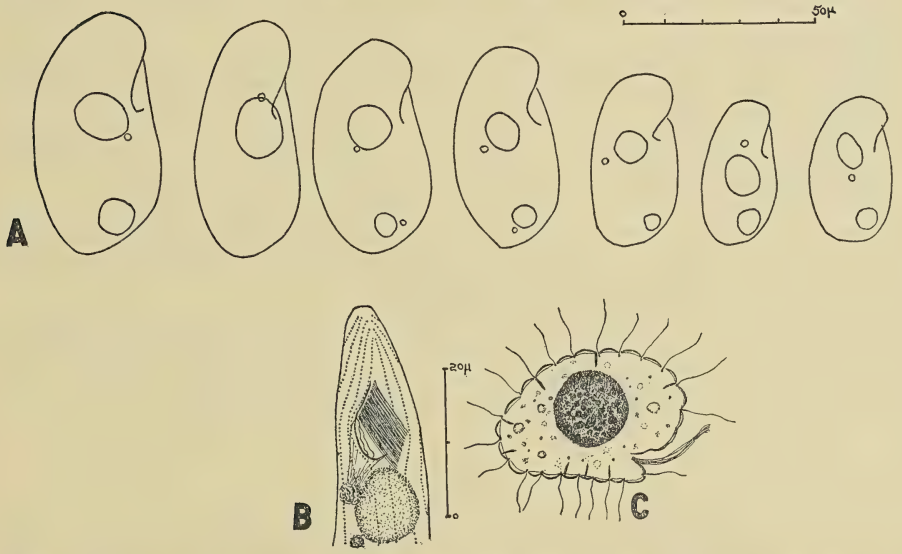

FIG. 12—*Entodiscus sabulonis* n. sp. A, showing range of variability for size, shape of body and nuclei. B, showing pattern of ciliary rows about peristome and some details of the cytostome. C, cross-section through region of peristome, showing number of striations, trichocysts (?), and peristomal membrane.

Diagnosis—Average length 52μ, width about 0.4 of length. Body flattened with both ends rounded. Cytostome located at base of a C-shaped depression in left anterior margin. Anterior region of peristome contains brush of 6 to 8 rows of cilia. Body cilia are arranged in 21 longitudinal rows. Spherical macronucleus central, accompanied by single micronucleus. Simple contractile vacuole. Posteriorly a long tactile filament is inserted to one side of rounded caudal region.

Description—Average length 52 (35 to 64)μ; average width 23 (18 to 26)μ. *E. sabulonis* is about 14μ in thickness and usually moves about on its flattened ventral surface; the dorsal surface being slightly convex. An examination of cross-sections of material fixed in F.W.A. (text-fig. 12C) demonstrated numerous rod-like bodies in the ectoplasm, suggestive of trichocysts. The presence of these trichocysts (?) was not detected during the examination of the living organisms.

Although the cytostome is well defined, lying at the base of a well-marked peristomal furrow, the pattern of the ciliary rows in the peristome has not been determined. There is evidently a thick brush of cilia anterior to the cytostome, but how these cilia are arranged and whether or not they are accompanied by membranes of some type, as there is some evidence to believe, the material at hand fails to demonstrate. Extending from the cytostome there is a very short cytopharynx. The food vacuoles were usually filled with bacteria or with cells from the intestinal mucosa of the host. This ciliate demonstrated a marked preference for spermatozoa obtained from its host, and could be seen packing them away in its food vacuoles with great avidity.

The contractile vacuole is rather constant in position, being found posteriorly on the same side as the cytostome. After fixation with Schaudinn's, if material is not thoroughly washed there is often a thin crust of yellow substance which forms about the contractile vacuole and marks it off from other vacuoles.

Posteriorly, the terminal filament is usually found in a position a little to the right of the center. The caudal bristle is rather stout and associated with a large basal granule. All observations point to this terminal filament as being capable of independent movement; hence it is often found pointing to the right or left of the line of the body axis.

The endoplasm is usually clear and undifferentiated, except for the numerous fat droplets which in the larger individuals so fill the cytostome that the other structures are obscured. Fat is best demonstrated by prolonged osmification, or through the use of Sudan III.

Hosts—E. sabulonis was found in abundance in all of the individuals of the two species of *Clypeaster* examined. Since the number of hosts available for examination was small (8 of *C. rosaceus* and 3 of *C. subdepressus*), knowledge of the degree of infestation is necessarily limited.

Undetermined Species

(Text-fig. 13)

In the course of this investigation observations have been made on a few ciliates which, though having characteristics in common with some of the species that are well defined, do not fit in with any of these well-established forms. Hence these species are described below in as much as the limited observations will allow, and for convenience will be designated by letters.

Form L—This is either a variety of *E. sabulonis*, or is a species closely related to it (Plate 2, fig. 7). Because of the lack of suitably preserved material the data concerning the cytostome and the number of striations is incomplete. The only material at hand consists of one OsO_4 glycerine jelly mount, together with notes covering some observations made on the living specimens. Form L differs from *E. sabulonis* in both size and shape. In general it is smaller and the body is cylindrical with the caudal spine pro-

jecting from a centrally located basal granule. It differs further in that the peristomal cilia are parallel to the body axis and not oblique with or perpendicular to it. The endoplasm is undifferentiated, except by the presence of great numbers of fat droplets and a few granules of glycogen. Conjugation was observed. This form was found in only one individual of *Clypeaster subdepressus*.

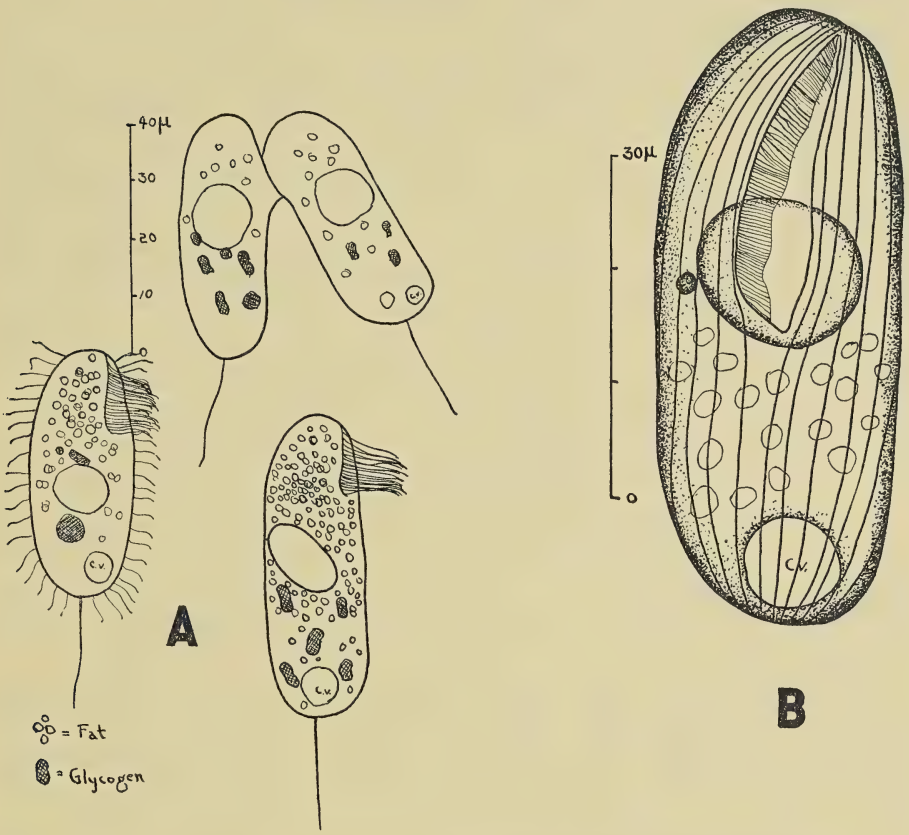

FIG. 13—Undetermined species: A, form L; sketches from life. B, form M; from a smear fixed in Shaudinn's.

FORM M—This species is cylindrical in shape ranging in size from 25 to 60μ long, width about 0.4 length. As to position of peristome and general body shape, this species is suggestive of *Cohnilembus cœci;* its chief difference from the latter being in the greater number of striations covering its surface. While *C. cœci* has 10 well-defined striations, form M is characterized by at least 18 or more. The stout body of form M in contrast to the slender figure of *C. cœci* relates it rather closely to *Anophrys vermiformis* common to *Lytechinus variegatus* of Beaufort, North Carolina.

Form M was found on smears taken from *Tripneustes esculentus* and *Lytechinus variegatus*. It was not studied in the living condition, because its presence was not suspected until long after this study was confined to fixed material.

TYPES OF CILIATE INFESTATION

It is not possible to form conclusive statements concerning the types of ciliate infestations found among the Tortugas echinoids. The data at hand is too fragmentary in regard to the forms dwelling in deep water. During the summer of 1933 very little dredging was done and only 14 specimens were examined from depths below 10 fathoms. Therefore the following conclusions are drawn tentatively:

A survey of table 2 will illustrate the wide spread of ciliates, both in relation to depth of water as well as to choice of hosts. From this data we may conclude:

1. That the greater number of species of ciliates are found infesting those hosts near the tide line.

2. Below 10 fathoms, the ciliate fauna is limited to *Crytochilidium bermudense, Anophrys elongata, Cohnilembus cæci* and form M; with *C. bermudense* being by far the most abundant form.

3. There is little evidence to indicate a rigid host specificity holding for the ciliates represented in this study, other than the following:

a—*Metopus rotundus* is limited to *Centrechinus antillarum.*

b—*Metopus brevicristatus* is limited to *Clypeaster rosaceus.*

c—*Metopus histophagus* is found only in *Clypeaster subdepressus.*

d—*Entodiscus sabulonis* is limited to the genus *Clypeaster.*

In contrast with the above host-parasite relationships, we have the almost universal distribution of *Cryptochilidium bermudense, Anophrys elongata, Cohnilembus cæci* and *Metopus circumlabens* among the hosts in shallow water.

4. While there may be a large group of ciliates wholly cosmopolitan in regard to their hosts, nevertheless each species of this cosmopolitan group demonstrates a definite host preferability. Thus judging from the abundance of individuals and the frequency with which a species is found in a particular host, it may be pointed out that:

a—*Tripneustes esculentus* is the preferred host of *Cohnilembus cæci,* for although the latter may be found in any one of four species of echinoids, it is always most abundant in the former.

b—*Cryptochilidium bermudense* and likewise *Anophrys elongata,* though common to all echinoids, are most abundant in *Lytechinus variegatus.*

Are the above observations indicative of relationships so long established that they have outgrown the bounds of "host specificity"; and, conversely, are those associations exhibiting rigid affinity for a particular host the more recently formed?

With the exception of very young specimens and all individuals of *Eucidaris tribuloides,* no sea-urchins were found to be completely devoid of ciliate infestants.

TABLE 2—*Relative frequency of ciliates infesting Tortugas echinoids*

Echinoid Host	Ciliates					
	Metopus circumlabens	Metopus rotundus	Metopus brevicristatus	Metopus histophagus	Cryptochilidium bermudense	Anophrys elongata
Shallow water (not over 2 fathoms)						
Lytechinus variegatus	2++				2++	2++
Centrechinus antillarum	86++	11+			40 - to ++	70 + to ++
Echinometra lucunter	14++				3+	10+
Tripneustes esculentus	4++				2++	5++
Clypeaster rosaceus	2-		8+		8--	2-
Clypeaster subdepressus	1--			3+	2--	
Eucidaris tribuloides						
Deep Water (10 to 50 fathoms)						
Lytechinus variegatus From 10 fathoms					12++	12++
Astrophyga magnifica From 48 fathoms					2++	2++

Echnoid Host	Ciliates						Total No. Specimens Examined
	Anophrys aglycus	Cohnilembus cæci	Entodiscus sabulonis	Cyclidium rhabdotectum	Form L	Form M	
Shallow water (Not over 2 fathoms)							
Lytechinus variegatus		2+				1-	2
Centrechinus antillarum	9++	22+		19 - to ++			86
Echinometra lucunter	3+	3-		6++			14
Tripneustes esculentus		22 + to +++		1+			22
Clypeaster rosaceus			8++	2-			8
Clypeaster subdepressus			3++		1++		3
Eucidaris tribuloides							26
Deep Water (10 to 50 fathoms)							
Lytechinus variegatus From 10 fathoms		1++				1++	12
Astrophyga magnifica From 48 fathoms							2

In above table the numbers refer to number of individual sea-urchins found to be infested with indicated ciliate; - or + refers to the relative abundance of the infestation; - = rare, + = abundant, and ++ = very abundant.

As to the nature of this endozoic association: If we define parasitism as a relationship through which one member is benefited to the detriment of the other, then these ciliates are clearly non-pathogenic, hence not parasites. If, on the other hand, we consider a parasite as an animal dependent for its existence upon its host, then obviously these ciliates are all parasitic for they have not as yet been shown to be capable of but more than a few days existence in sea-water removed from their normal habitat. The writer prefers to think of this relationship as one of commensalism in which the ciliates act as scavengers, eating bacteria, diatomes, small bits of algæ and often cellular débris derived from the mucosa of the hosts intestine—commensals in the sense that the infusoria are benefited by the association without harming their host.

Metopus histophagus and *Entodiscus sabulonis* are the only members of this group demonstrating consistently food vacuoles filled with material of animal tissue origin. *M. rotundus* has been observed ingesting three or four *Anophrys elongata* in succession; and other ciliates have demonstrated a marked liking for spermatozoa, filling many food vacuoles with the latter when given the opportunity. Despite their carniverous tastes, the above ciliates have never been observed as the cause of lesions in the alimentary tract.

Since an infestation has never been followed throughout the life span of a host, there is no data on the time and mechanism of the initial infestation. It has been observed, however, that liquid dripping from the anus of an adult will often contain numerous ciliates, representative of the fauna of the host, which suggests a mode of transmission from one individual to the alimentary tract of another, through the living medium.

An examination of the ciliate fauna infesting members of the genus *Clypeaster* (table 2) shows that frequently one encounters there, in very few numbers, ciliates more commonly associated with other echinoids. Thus one may find an occasional *Metopus circumlabens*, *Cryptochilidium bermudense* or *Cyclidium rhabdotectum*. The individuals seem to be vagrants, in an environment unsuitable to their needs for they are never found there in great numbers. Similar instances were observed at Beaufort, where, upon examination of the centrifuged intestinal fluid from numerous *Arbacia*, never more than one or two individuals of *Cryptochilidium bermudense* were found. Although these two cases mentioned belong to entirely different categories, in so far as *Clypeaster* is the host for a well-defined group of ciliates, while *Arbacia* entirely lacks such a relationship, they both represent examples of chance associations. We are still at a loss in offering any explanation for the remarkable resistance which *Eucidaris tribuloides* of Tortugas and members of the Genus *Arbacia* at Beaufort and Woods Hole display to infestation by ciliates, even though they may live near or among echinoids known to carry a heavy infestation. Moreover, our knowledge of the factors operating which enable these commensals to exist in an

environment positively lethal for free-living marine ciliates is very meager and is based entirely on the studies of Jacobs (1914). It was he who first noted that, "Free-living forms (*e.g.* Foraminifera, etc.), which are taken in with the food, are invariably dead before the second coil (of the intestine) is reached." None of the common saprozoic marine ciliates were ever encountered in the mid-gut, although they might abound among the fecal pellets, etc., in the bottom of an aquaria containing sea-urchins, all heavily infested.

OTHER ORGANISMS INFESTING ECHINOIDS

(Text-fig. 14)

Although the writer did not find amebæ among the protozoa infesting echinoids, as reported by Jacobs (1914), occasional infestations of flagel-

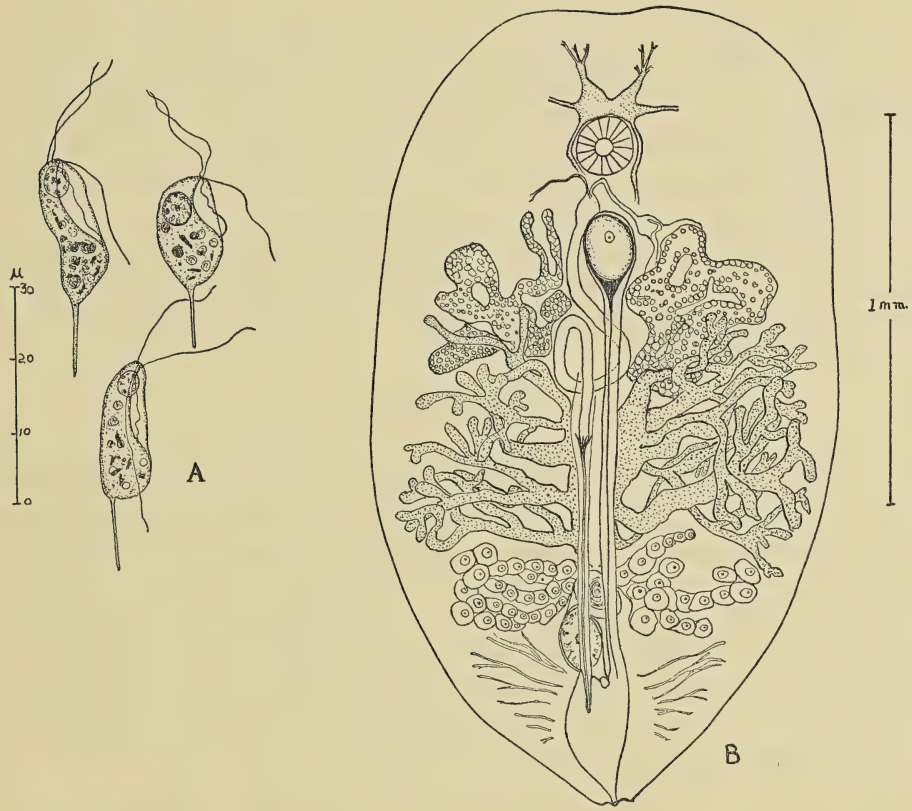

FIG. 14—Other organisms associated with sea-urchins: A, *Chilomastix echinorum* n. sp., from *Tripneustes esculentus*: B, *Syndesmus* sp. from *Centrechinus antillarum*; ventral view showing principal characteristics.

lates were observed. In the intestinal cæcæ of two large *Tripneustes esculentus* gathered from the warm waters of the flats off Bird Key were found swarms of *Chilomastix echinorum* n. sp. (text-fig. 14A). Compared to *C. mesnili*, these flagellates were rather large. Their length varied from 26 to

32μ. The cytostome is located on one side near the anterior end and contains an undulating membrane. The cytoplasm was usually filled with food vacuoles. Food consisted of bacteria, judging from their contents. Anteriorly, three flagella arise from blepharoplasts located just beneath the ectosarc. The nucleus is spherical and lies close to the anterior end. Posteriorly, the body extends in a long spine-like projection suggestive of an axostyle. In both of the observed cases, the hosts appeared in good condition and the gut contents were in no way unusual, except for these swarms of flagellates. In each instance, large numbers of *Cohnilembus cœci* accompanied these flagellate infestations, a point of interest as ciliates and flagellates are rarely found together in such numbers.

In about 5 per cent of the *Centrechinus antillarum* examined there were found in the cœlomic cavity, fastened to the outer wall of the intestine, from 5 to 16 small Rhabdocœla. These Turbellaria were a bright red in color, measuring 1 to 1.5 mm. and morphologically are related to *Syndesmus echinorum* Franc. (Shipley, 1901), parasitic in *Echinus esculentus* from the English Channel. This Tortugas rhabdocele (text-fig. 14B) differs from *Syndesmus echinorum* in the arrangement of its shell glands as well as in the general morphology of the posterior region. This difference is especially noticeable in the details of the copulatory apparatus.

A few small immature nematodes were noted from time to time in the guts of various sea-urchins. These were never found in such abundance or with sufficient constancy to suggest there being anything but the result of a chance relationship.

DISCUSSION

The greatest interest in this study has been to disclose the origin and the factors which give rise to associations of endoparasitism—or still better termed, endocommensalism. It is of importance today in the field of parasitology to determine the factors and conditions which induce an organism to become wholly dependent upon an endozoic habitat. Perhaps through an examination of taxonomic data, showing relationships between species, and geographical distribution, which will show the spread of the infestation, we may begin an introduction to the solution of some of these problems—a solution to be found no doubt only through the use of carefully planned experimentation involving controlled infestations.

QUESTIONS OF TAXONOMY

As a foundation for all of our work it goes without saying that there must be a sound morphological basis upon which all the experimental superstructure must rest. While the first objective of Taxonomy is that of classification, coequal with it is the demonstration of relationships among structurally comparable forms. Clearly, when dealing with protozoa the first objective is the more easily attained; the second is doubly difficult because of

the great diversity shown in the complexity of such structures as the cytostome or the general body configuration and in the painstaking technical procedures and repeated observations on a great variety of preparations, necessary to clarify definitely our knowledge of the actual structures present—many of which are near the limits of resolution of our optical equipment. In view of these latter difficulties, the overcoming of which is a matter of years of study, the policy has been adopted of considering the gross microscopical features of each ciliate and with as little recourse as possible to the study of minute detail, giving taxonomic status to each. In this manner the infestation as a whole may be presented for consideration in the broad terms of parasitology.

The only group which presented any real difficulty in the determination of morphological characteristics was the *Anophrys-Cohnilembus* forms, whose position is determined largely on the possession of one or two membranes fastened to the right margin of the peristome. With the excellent analysis by Hoare (1927) of the genus *Cohnilembus* there is now no doubt concerning its characteristics. However, with two peristomal membranes coming from the same margin, it is usually very difficult to demonstrate both, for the two appear usually as only one because of the close proximity of their basal insertions. Therefore, unless two definite membranes could be demonstrated, the species in question was referred to the genus *Anophrys*.

Cohnilembus cœci is interesting because of its close affinity with *Anophrys echini* Di Mauro 1904. The writer's material does not afford a critical analysis of *A. echini,* and until fresh material is examined it will suffice to point out here the great similarity of these two forms.

The structural similarities of the members of the genus *Metopus* suggest recent modifications which have been of degree in development, rather than the acquisition of new structures, these changes being confined to ciliary pattern and the general plan of the body shape.

Great diversity is shown among the *Cohnilembus-Anophry*-form ciliates, so much so that the nearness in relationship is not so apparent as among the members of genus *Metopus*.

It is interesting to note that *Cyclidium rhabdotectum,* in addition to serving as a commensal in one animal, is itself subjected to such an association by the bacteria which cover its pellicle.

GEOGRAPHICAL DISTRIBUTION

As assembled in table 3, the data from Bermuda, Beaufort and the Dry Tortugas give us the beginning of an idea of the distribution of these ciliates in localities bordering the Gulf Stream. Of these three regions, the area about the Dry Tortugas is by far the richest in its fauna of sea-urchins and their commensals.

Are the similarities and differences in the distribution of ciliates suggestive of a time relationship in the development of these associations? Since,

TABLE 3—*Distribution of ciliates among sea-urchins from localities bordering the Gulf Stream as reported by various workers.*

Locality	Echinoid Host	Ciliates							
		Metopus circumlabens	Metopus rotundus	Metopus brevicristatus	Metopus histophagus	Cryptochilidium bermudense	Cryptochilidium echinometris	Anophrys elongata	Anophrys aglycus
Bermuda	Lytechinus variegatus					Bray '25 Bigger '32		Bigger '32	
	Centrechinus antillarum	Bigger '32 Lucas '34	Lucas '34						
	Echinometra lucunter	Bigger'32 Lucas '34					Bigger '32	Bigger '32	
Beaufort [2] N. C.	Lytechinus variegatus					Bray '25 Powers '33	Powers '33 [1]		
Dry Tortugas [3]	Lytechinus variegatus	Jacobs '14 Powers '35				Jacobs '14 Powers '35		Jacobs '14 Powers '35	
	Centrechinus antillarum	Jacobs '14 Powers '35	Powers '35			Jacobs '14 Powers '35		Jacobs '14 Powers '35	Powers '35
	Echinometra lucunter	Jacobs '14 Powers '35				Jacobs '14 Powers '35		Powers '35	Powers '35
	Tripneustes esculentus	Powers '35				Powers '35		Powers '35	
	Astrophyga magnifica					Powers '35		Powers '35	
	Clypeaster rosaceus	Powers '35 (rare)		Powers '35		Powers '35 (rare)		Powers '35 (rare)	
	Clypeaster subdepressus	Powers '35 (rare)			Powers '35	Powers '35 (rare)		Powers '35 (rare)	

[1] Further study shows that this may be an aberrant form of *Cryptochilidium bermudense.* [2] *Arbacia punctulata* from Beaufort was also examined, but was found lacking in any ciliates. [3] *Eucidaris tribuloides* from Tortugas was completely negative regarding any ciliate infestation.

TABLE 3—(*Continued*)

Locality	Echinoid Host	Ciliates						
		Anophyrs vermiformis	Cohnilembus cæci	Colpoda fragilis	Entodiscus sabulonis	Cyclidium rhabdotectum	Form L	Form M
Bermuda	Lytechinus variegatus							
	Centrechinus antillarum							
	Echinometra lucunter							
Beaufort[2] N. C.	Lytechinus variegatus	Powers '33, '35	Powers '35	Powers '33, '35				
Dry Tortugas[3]	Lytechinus variegatus		Powers '35					Powers '35
	Centrechinus antillarum		Powers '35			Powers '35		
	Echinometra lucunter		Powers '35			Powers '35		
	Tripneustes esculentus		Powers '35			Powers '35		Powers '35
	Astrophyga magnifica							
	Clypeaster rosaceus				Powers '35	Powers '35 (rare)		
	Clypeaster subdepressus				Powers '35		Powers '35	

of the 15 ciliates listed (in table 3), *Cryptochilidium bermudense* heads the list both in prevalence and in numbers of individuals, are we justified in holding it to represent the oldest member of the association in the entire group?

If we consider the relationship of ciliates in reference to locality:

1. We have in common with all of this area only *C. bermudense.*
2. In common with the two regions more definitely in the Gulf Stream (Bermuda and Tortugas) we have: *C. bermudense, Metopus circumlabens, M. rotundus* and *Anophrys elongata.*
3. *Cohnilembus cœci* is the only species represented at Tortugas and Beaufort, but not reported from Bermuda.

Remaining are three groups: (1) Those limited to Bermuda: *Cryptochilidium echinometra*; (2) Those limited to Beaufort: *Anophrys vermiformis* and *Colpoda fragilis*; and (3) Those limited to the Tortugas region: *Metopus brevicristatus, M. histophagus, Anophrys aglycus, Entodiscus sabulonis, Cyclidium rhabdotectum,* and forms L and M.

Can we explain the presence of those species common to two or more localities by saying that they represent associations formed in the original echinoid habitat before land movements distributed their host over a wide area; and that those species common to one locality represent associations formed in the particular region after the establishment of the echinoid? It would help us greatly in the understanding of this range if we had data covering the distribution of ciliates among the echinoids of the Lesser Antilles, for here is the probable site from which all of the cosmopolitan members of the infestation have spread, using the Gulf Stream as a vector.

While the supposition that the Lesser Antilles is the locus of this infestation may hold for those forms peculiar to the Caribbean, the findings of Yagiu (1935) demonstrate that the more cosmopolitan forms of the Gulf Stream echinoids occur also in the littoral sea-urchins about Japan. When the proposed work of Lynch (1929) is finished, the data will be more complete concerning the distribution of, as well as the relationships among, these ciliates infesting the Pacific sea-urchins.

Yagiu (1934) has described three species of *Cryptochilidium* which, with the forms already known, make a total of seven species in this genus, all of which infest echinoids. These seven species form a rather well-graded series in which the cytostome remains almost constant at about the level of the macronucleus, speciation being therefore a result of differentiation with regard to the peristomal structures and extensions of the region anterior to the cytostome.

The echinoids are of far more ancient origin than the terrestrial ruminants, yet consider how numerous are the ciliates infesting the latter (some contain over 100 different species of ciliates most of which are exceedingly complex and bizarre).

It is supposed that life for these marine ciliates infesting an aquatic host is far easier than that existence common to protozoa infesting land-dwelling animals, and because of this the ciliates of sea-urchins show no more specialization of their ectosarc than do the free-living Holotricha.

SUMMARY

1. Twelve species of ciliates are described which infest the alimentary tract of seven species of sea-urchins from the region about the Dry Tortugas. Eight of the twelve are new.

2. Only five of these twelve species show any marked host specificity; *Metopus rotundus* is limited to *Centrechinus antillarum*; *Entodiscus sabulonis* infests only members of the genus *Clypeaster*; *Metopus brevicristatus* is limited to *Clypeaster rosaceus*; *Metopus histophagus* and form L are limited to *Clypeaster subdepressus*.

3. The remaining seven species all show a definite host preference, although they may be cosmopolitan in their distribution: *Centrechinus antillarum* is the preferred host of *Metopus circumlabens*; *Lytechinus variegatus* is the preferred host of *Cryptochilidium bermudense, Anophrys elongata* and form M; *Tripneustes esculentus* is the preferred host of *Cohnilembus cœci* and *Anophrys aglycus*; *Echinometra lucunter* is the preferred host of *Cyclidium rhabdotectum*.

4. All of these ciliates are associated with their host when the latter are found occurring near the tide line.

5. In sea-urchins taken below a depth of ten fathoms, only four species of ciliates are found: *Cryptochilidium bermudense, Anophrys elongata, Cohnilembus cœci* and form M.

6. The nature of this infestation is one of endocommensalism, there being no present evidence to indicate pathogenic tendencies for any of its members.

7. The name *Cyclidium stercoris* is proposed for *Uronema sociale* Powers 1933, which must be suppressed because it is a homonym of *U. sociale* Penard 1922.

8. Besides ciliates, *Chilomastix echinorum* was found infesting the intestine of an occasional *Tripneustes esculentus*; also, from the cœlom of *Centrechinus antillarum* a number of red Rhabdocœla were obtained, obviously related to the genus *Syndesmus*.

9. Data concerning the geographical distribution of the ciliates infesting sea-urchins from the localities of Beaufort, North Carolina, Bermuda and Tortugas have been summarized. It is suggested that the center of this infestation will be found in the sea-urchins from the region of the Lesser Antilles and that this infestation has been carried northward along with its host, through the agency of the Gulf Stream.

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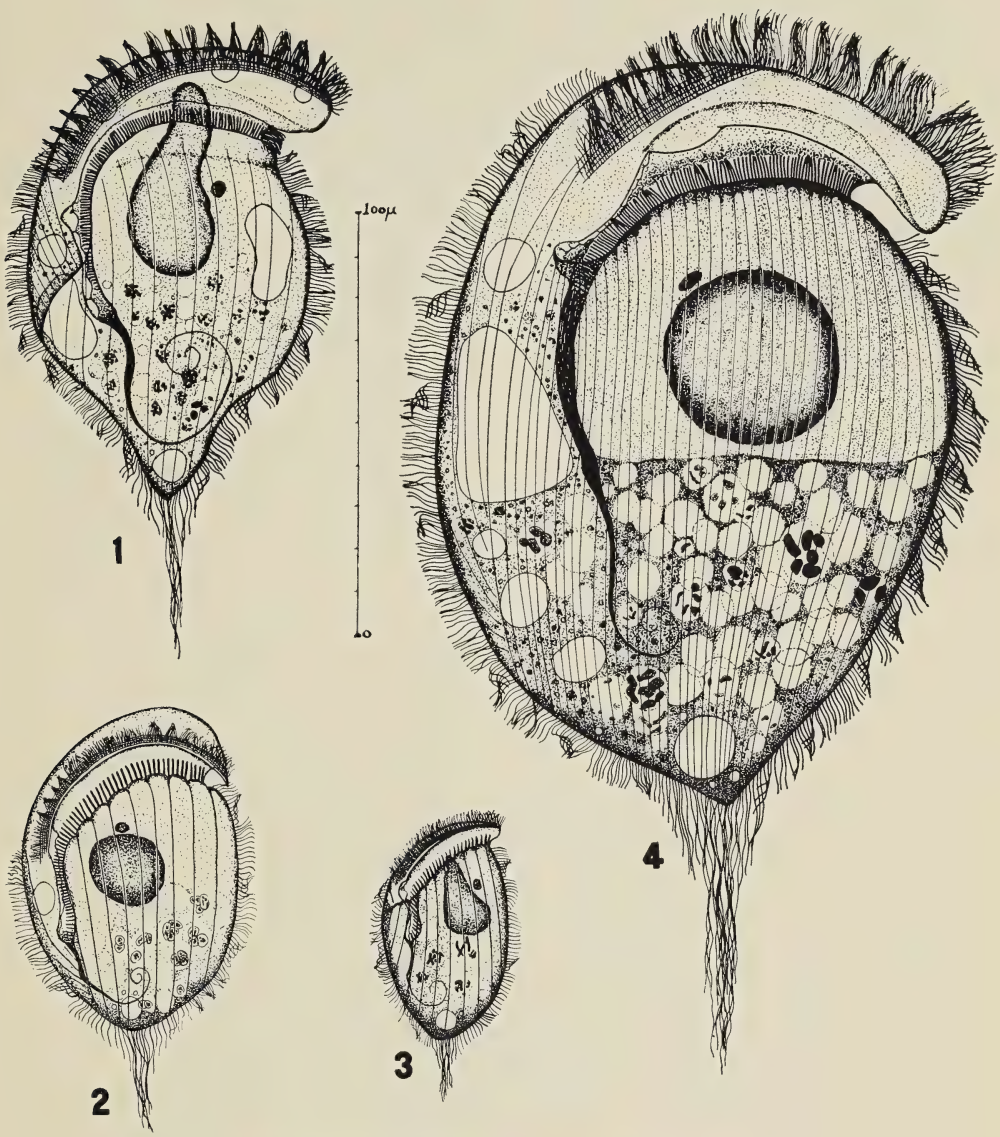


Fig. 1—*Metopus circumlabens* Biggar and Wenrich
Fig. 2—*Metopus histophagus* n. sp.
Fig. 3—*Metopus brevicristatus* n. sp.
Fig. 4—*Metopus rotundus* Lucas.

Figures were drawn, with aid of camera lucida from representative individuals, to scale as indicated on plate.

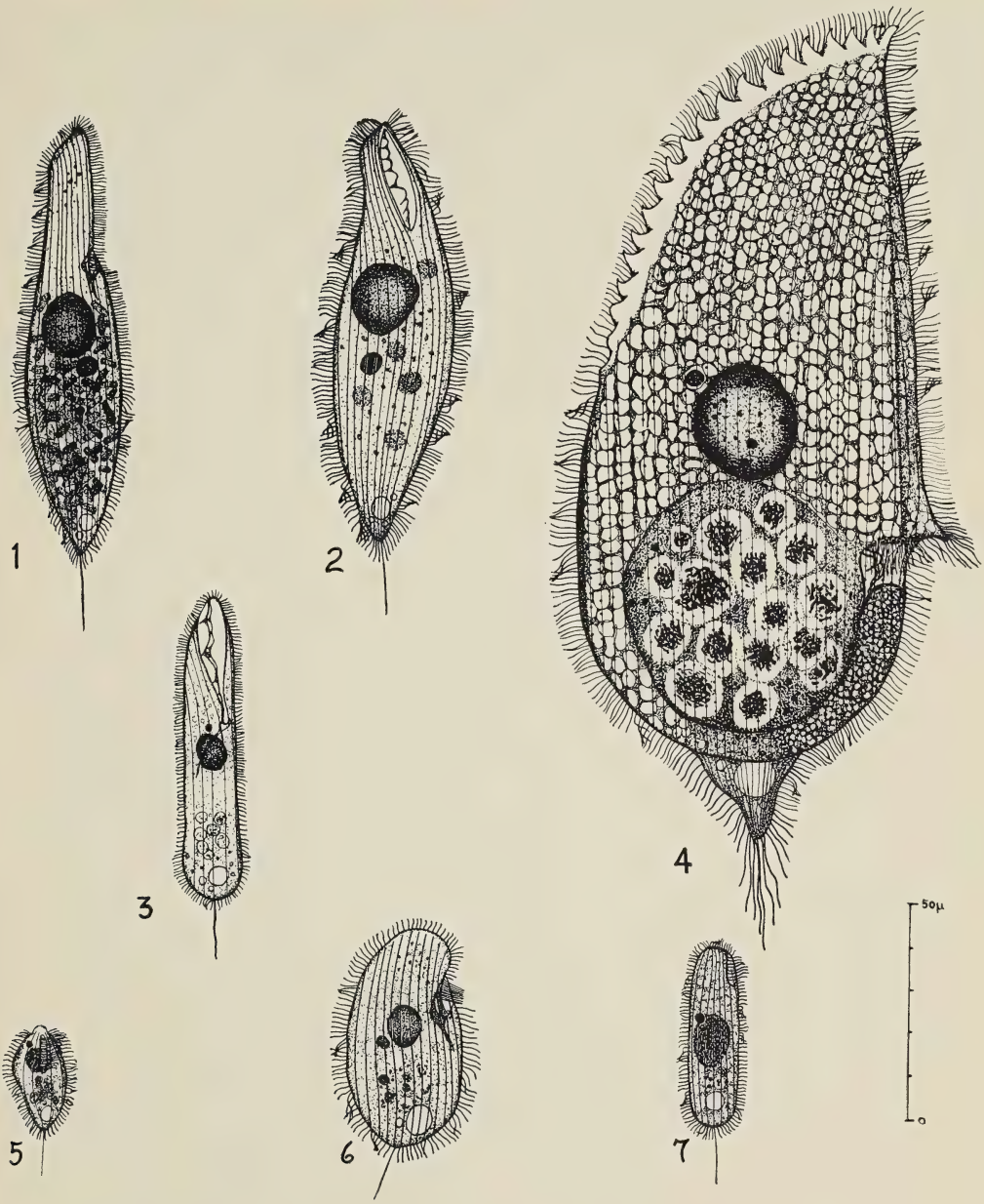


Fig. 1—*Anophrys elongata* Biggar and Wenrich. Note included glycogen granules.
Fig. 2—*Anophrys aglycus* n. sp.
Fig. 3—*Cohnilembus caeci* n. sp.
Fig. 4—*Cryptochilidium bermudense* Biggar and Wenrich. Ventral view.
Fig. 5—*Cyclidium rhabdoctectum* n. sp. For illustration of bacteria which cover the surface see text fig. 11A.
Fig. 6—*Entodiscus sabulonis* n. sp.
Fig. 7—Form L, an undetermined species.

Figures were drawn, with aid of camera lucida from representative individuals, to scale as indicated on plate.

PAPERS FROM TORTUGAS LABORATORY, VOL. XXIX

XII

PARASITIC COPEPODS FROM THE DRY TORTUGAS

By Charles Branch Wilson

With six plates

[Issued December 9, 1935.]

PARASITIC COPEPODS FROM THE DRY TORTUGAS

The two collections of parasitic copepods contained in the present paper were made by Dr. Harold W. Manter of the University of Nebraska and Dr. O. L. Williams of the College of the Pacific. The investigations of these two scientists, carried on at the Marine Laboratory of the Carnegie Institution in the Dry Tortugas, involved the handling of a large number of the local fishes. In addition to the specific objects of investigation it was soon noted that the fish were more or less infested with parasitic copepods and isopods. These were carefully removed and preserved and in this way quite a collection was accumulated by each of the investigators and turned over to the present author for identification. The specimens proved to be excellently preserved and had been removed from their hosts with such skill that the attachment organs were all intact. It is a great pleasure to work with such material and sincere thanks are hereby returned for the privilege.

Upon identification, seven of the species have proved to be new to science, two others have been made the types of new genera, and fully developed adults of both sexes have been obtained for another species which was originally founded upon an immature female. The other species have been obtained before, either in the waters around the Dry Tortugas, the Bahamas, the Bermudas or the West Indies. But for the majority of them the present locality is new, and in a number of instances the host is also new.

In a recent paper [1] the statement was made that the specimens described by Dana 80 years ago as a new genus and species under the name of *Specilligus curticaudis* are really the copepodid stage of the male of *Pandarus cranchii.* Proof of that statement can be here given and a series of figures presented showing the gradual transition from the copepodid stages into the adult. The new species have been fully described and figured, while the others are merely listed with such explanatory notes as seem desirable.

ARGULOIDA

Argulus laticauda Smith

———. Wilson, Proc. U. S. Nat. Mus., vol. 25, p. 705, pl. 10, figs. 5–9, 1902.

Occurrence—Two females were taken from the outer surface of the spotted jewfish, *Promicrops itaira,* by Dr. Manter. This is a new locality as well as a new host for this parasite, and is the first record of the species outside the Woods Hole area. It certainly does occur this far south and, therefore, may be looked for with considerable probability around the Bahamas and the Bermudas.

[1] Smithsonian Miscellaneous Collections, vol. 91, No. 19, p. 1.

CALIGOIDA

Caligus aliuncus Wilson

———. Wilson, Proc. U. S. Nat. Mus., vol. 28, p. 576, pl. 9, figs. 103–110, 1905.

Occurrence—Two females were found upon the outer surface of the bonito, *Euthynnus alleteratus*, by Dr. Manter and are the first to be obtained since the original discovery. The species was founded upon a single female captured while swimming freely at the surface under an electric light; there were no data as to locality or host and the ovisacs were broken. The present specimens give us both locality and host, supplement the structural details and confirm the original description.

Female—The larger of the two specimens is 7.60 mm. long, with perfect ovisacs 4.60 mm. in length. The posterior lobes on the genital segment are proportionally longer and more pointed than in the original specimen, but otherwise the general description of the body is the same. Furthermore these lobes carry two pairs of rudimentary legs, a fifth pair on the lateral margins at the bases of the lobes and a sixth pair on the ventral surface near the tips of the lobes. Both pairs were either absent from the original specimen or escaped detection in the limited examination possible. The attachment hooks, wrongly designated as first maxillæ in the original description and said to be lacking, are really present just behind and outside of the bases of the second antennæ. But they are situated in a depression of the ventral skin and are so minute as easily to escape detection.

The smaller of the specimens was only 5 mm. long and is evidently in a copepodid stage since the abdomen has but three segments, with the third one only partially separated. The male of the species still remains unknown.

Caligus irritans Heller

———. Wilson, Proc. U. S. Nat. Mus., vol. 44, p. 210, pl. 27, figs. 71–80, 1913.

Occurrence—A single female was taken from the mouth of a grey snapper, *Lutianus griseus*, a new host and locality for the species. The Jamaica specimens were found on the gills of a red snapper, while Heller's original types came from a sea bass on the Brazilian coast.

Caligus suffuscus Wilson

———. Wilson, Proc. U. S. Nat. Mus., vol. 44, p. 219, pl. 30, figs. 107–115, 1913.

Occurrence—A single female was taken from the gills of the jolthead porgy, *Calamus bajonado*, by Dr. Manter, while Dr. Williams found one female on the outer surface of the hogfish, *Lachnolaimus maximus*, and another female on the lip of the barbero, *Acanthurus cœruleus*. All three of these hosts as well as the locality are new for the species.

Lepeophtheirus dissimulatus Wilson

———. Wilson, Proc. U. S. Nat. Mus., vol. 28, p. 631, pl. 22, figs. 259–272, 1905.

Occurrence—A single male was taken from the outer surface of the trunkfish, *Lactophrys triqueter*, both the host and locality being new.

Paralebion elongatus Wilson

Plate 1, figs. 6-12; Plate 2, fig. 13

———. Wilson, Proc. U. S. Nat. Mus., vol. 39, p. 628, pl. 66, figs. 13–21, 1911.

Occurrence—One female and two males were found on the gills of a short-nosed shark, *Hypoprion brevirostris*, by Dr. Manter. This is the first record of the species since it was established upon specimens obtained from an unidentified shark in Chesapeake Bay in 1905. Both sexes in the Tortugan specimens are larger than the types and differ in the following particulars.

Female—The carapace and genital segment (fig. 6) are each longer than in the original types, the rudimentary plates on the fourth segment have a different shape, the first maxillæ are straight and not curved, while the basal portion of the double furca is relatively smaller and the spine at the tip of each branch is lacking. Total length of female 10.80 mm. Carapace 4 mm. long, 4.30 mm. wide.

Male—The fourth (free) segment has a conspicuous wrinkle where it joins the carapace, and there are no traces of dorsal plates. The first maxillæ (fig. 8) are stouter and corrugated along their inner margins, and the maxillary palps at their bases are much larger, each 2-segmented and tipped with 3 setæ. The basal portion of the double furca (fig. 10) is here reduced to mere rudiments on either side of the terminal portion. The fourth legs (fig. 12) are powerfully developed, with 5 strong claws, the terminal one exceptionally long. The bases of all these claws and the outer margins of the segments near the bases are fringed with stiff hairs. Total length of male 7.25 mm. Carapace 3.54 mm. long, 3.87 mm. wide.

Remarks—The differences enumerated above are such as would naturally be expected in more mature specimens and do not affect the identity of the species. In 1892, P. J. van Beneden described and figured a new genus and species under the name *Papulina flores*. He claimed to have both sexes, but if his so-called male be compared with figure 6 it will be evident that it was really a young female of the present species without egg strings. In further proof of this statement twelve specimens of Beneden's species, including the real male, were recently obtained by the National Museum and will be described in the near future.

Dentigrips curtus Wilson

———. Wilson, Proc. U. S. Nat. Mus., vol. 44, p. 222, pl. 32, figs. 127–135, 1913.

Occurrence—The original specimens upon which this new genus was founded were taken from the mouth and gills of the poison grouper, *Mycteroperca venenosa*, at the Bermuda Islands. Five females were obtained here at the Dry Tortugas by Dr. Manter from the gills of the same host, and twelve females by Dr. Williams from the outer surface of the body. Evidently the male is less common than the female and still remains unknown.

Anuretes parvulus Wilson

Plate 1, figs. 1-5

———. Wilson, Proc. U. S. Nat. Mus., vol. 44, p. 223, pl. 33, figs. 139–147, 1913.

Occurrence—This species was founded upon a single female from the gills of a black angelfish, *Pomacanthus arcuatus*, captured here at the Dry Tortugas. The present collections include another female from the same host, seven specimens, including both sexes, from the gills of a spotted angelfish,

Angelichthys bermudensis, by Dr. Manter, and three females from the latter host by Dr. Williams. Since the original type female is proved to have been incompletely developed, while the present specimens include two females with ovisacs and a male, a new description and additional figures are here presented.

Female—It will be noted that the fused posterior body in the fully grown female (fig. 1) is considerably larger and of a different shape from that of the type specimen. The carapace also is not narrowed as much anteriorly, its lateral posterior lobes are more broadly rounded, and the median lobe is relatively smaller. In addition to the two matured females the present collection contained several development stages. One of the latter was an almost exact counterpart of the original type, while in another the posterior body is shown in figure 2, the abdomen plainly visible in dorsal view.

Evidently the abdomen is at first separated from the genital segment and the two are on the same level. Then the genital segment gradually thickens and absorbs the abdomen more and more completely, the caudal rami and the remnant of the abdomen remaining nearer the dorsal surface. This increase in the size of the genital segment also pushes its anterior surface forward. The fourth segment, caught between the genital segment and the median lobe of the carapace, is squeezed so thin that it almost disappears in dorsal view. This decrease in length is compensated by an increase in width until the segment protrudes on either side through the bases of the fourth legs. The increase of the genital segment is due in part at least to the large bulk of the eggs, as can be seen in figure 3, which is a little more highly magnified than figure 1. Each egg string is one-half longer than the genital segment and contains six eggs, the first and last nearly spherical, while the four intermediate ones are considerably flattened.

The fourth leg (fig. 4) shows a structure quite different from that of *Caligus* and *Lepeophtheirus,* especially in the size and arrangement of the segments and spines. The proximal segment is enlarged a little on its outer margin at the base; the second and third segments are very short, triangular and unarmed; the distal segment is almost as long as the proximal, with three spines at its tip. The inner spine is slender, moderately curved and longer than the segment; a second spine outside of it is half as long, while the third spine is still shorter and on the outer margin of the segment behind the bases of the two terminal spines. In figure 2 two pairs of leg rudiments can be seen, the fifth pair finger processes, each tipped with 3 setæ, the sixth pair 3 setæ only. As the genital segment increases in size the fifth pair migrate on to the ventral surface and become invisible dorsally.

Male—Cephalothorax almost orbicular, only a little longer than wide, the lateral areas very wide, the posterior lobes broadly rounded (fig. 5). The posterior sinuses are much deeper than in the female and the median lobe between them is much narrower and definitely widened posteriorly. The eyes are a little farther forward and not as prominent; in fact they are so completely buried in the tissues of the head as to be hardly visible. The fourth segment is long and narrow, but considerably thickened through the bases of the fourth legs. The genital segment is ovate, as wide as long and one-third as wide as the cephalothorax. It is contracted into a narrow neck anteriorly where it joins the fourth segment and has sinuous lateral margins with two pairs of leg rudiments visible dorsally. The sixth pair is at the

posterior corners of the segment and the fifth pair on the lateral margins in front of the corners. Each leg in both pairs is made up of 3 setæ only, without any trace of processes.

The abdomen is 1-segmented, wider than long and fully visible in dorsal view; the caudal rami are longer than the abdomen and somewhat divergent, each tipped with 4 setæ, 3 of which are longer than the ramus and abdomen combined. The basal segment of the first antenna has a row of 3 setæ on the dorsal surface in addition to those on the anterior margin. The second antennæ are much stouter than those of the female, with stronger claws. The first maxillæ are well-defined straight spines similar to those of *Caligus*, but apparently without palps. The maxillipeds are larger and stouter than in the female, but otherwise the same, and the swimming legs show no sexual modifications. Total length of male 1 mm. Carapace 0.71 mm. long, 0.69 mm. wide.

Remarks—This is the first male to be obtained for this genus, and together with the added details for the female will serve to differentiate the genus clearly from *Caligus* and *Lepeophtheirus*, where it was placed by early investigators. Since both sexes can now be included in the genus diagnosis it will be seen that Heller was fully justified in establishing his new genus. The new male has been given Cat. No. 64038 U. S. National Museum.

Pandarus cranchii Leach

Plate 5, figs. 58-70; Plate 6, fig. 71

———. Leach, Dictionnaire des sciences naturelles, vol. 14, p. 535, 1819. Wilson, Bull. 158, U. S. Nat. Mus., p. 435, fig. 273, 1932.

Occurrence—Two females and a male of this species were taken from the outer skin of a short-nosed shark, *Hypoprion brevirostris*.

The male is identical with the one described by Dana 80 years ago, which he made the type of a new genus and species, *Specilligus curticaudis*. He found no females to complete his genus and none has been discovered since, although many specimens of the males have been obtained. It has been suggested by several authors that Dana's specimens, instead of constituting a new genus, were probably the males of some genus whose females had already been described. Since their structural details corresponded fairly well with those of both sexes of *Nesippus*, they were provisionally referred to that genus by the present author in 1907 (Proc. U. S. Nat. Mus., vol. 33, p. 434). At that time the male of but a single *Nesippus* species was known, the so-called males of two other species proving to be immature females. There was thus nothing to indicate how much or in what way the males of different *Nesippus* species might differ among themselves. Since then the males of four other *Nesippus* species have been described and figured. These agree with one another in every generic characteristic, and they all differ so consistently from the *Specilligus* male that the latter can no longer be admitted to the same genus with them.

Specilligus, therefore, again becomes an outcast without definite assignment, but fortunately it can be relocated at once and with probable certainty. While these Tortugas specimens were being identified and at the very time the present species was up for consideration, there came one of those opportune coincidences that occasionally surprise the investigator. From a shark caught for the Ocean Leather Co. near Nassau in the Bahama Islands nine parasites were obtained and sent to Dr. Ulric Dahlgren of

Princeton University, who transmitted them to the present author for identification. They proved to be two young females and seven young males of *Pandarus cranchii* and the males formed a regular development series.

Their lengths ranged from 5 to 8 mm.; the three smaller ones were typical *Specilligus* males, while the four larger ones were just as typical *Pandarus* males. Figures 58 to 62 are dorsal views of five of these males, all drawn to the same scale, which is shown with figure 62. Figures 63 to 67 show the fourth legs of the same males in regular order and with the same magnification. That these are really different stages in the development of the same species is evident from the following considerations.

1. They are all juvenile stages found together upon the same shark, and the females and larger males are certainly *Pandarus cranchii.*

2. The *Specilligus* males have been repeatedly captured while swimming freely in the tow, but there are no records of adult males of *Pandarus cranchii* taken in the same manner. This is exactly what would be expected of development stages in contrast with adult forms.

3. In the series here presented there is no trace of spermatophore receptacles in the three *Specilligus* forms, nor has anything of the sort been mentioned or figured by any author who has described them. The first trace of such receptacles is found in figure 60, and this male may be regarded as the first adult form. If the *Specilligus* males were really adults, as was assumed by Dana and all subsequent investigators, spermatophore receptacles ought to be at least partly visible.

4. In figure 58 each fifth leg is represented by a minute spine in a sinus near the distal end of the lateral margin of the genital segment. In figure 59 which is also a *Specilligus* form, sixth legs first appear as small processes at the posterior corners of the same segment. Both pairs of leg rudiments are subsequently further developed in figures 60 to 62 and 71.

5. In figure 58 the abdomen is really two-segmented, although the basal segment is so short that it has hitherto been regarded as merely a fold of the joint between the genital segment and the abdomen. The subsequent figures of the series, however, clearly show that it is to be regarded as the beginning of a second or basal segment.

6. The different stages in the development of the fourth legs are clearly shown in the series, beginning with the first *Specilligus* form in which both rami are undivided, and concluding with the adult forms in which both rami are distinctly 2-segmented.

7. The conspicilla, so prominent in the *Specilligus* forms, disappear entirely on transformation into the adult form. But the true eyes remain prominent until they more or less disappear under the tissues of the cephalothorax. The juvenile females have prominent eyes exactly like those of the males, which also disappear with maturity. As far as can be determined by an external examination, the conspicilla have no connection with the real eyes but are modifications of the carapace which are sloughed off with the moult into the adult stage.

8. The accessory lobes on the posterior margin of the cephalothorax inside of the posterior lobes can be traced as they gradually increase in size in the successive stages of development.

9. The maxillipeds in this Bahaman series as well as in the three Tortugan specimens are all of the forceps type (fig. 69) and just alike in both sexes. In the fully grown male (10 to 12 mm. long), however, the forceps has usually disappeared and been replaced by a claw (fig. 68).

From these considerations it seems evident that Dana's *Specilligus curticaudis* is neither a valid species nor genus, but is rather the male of *Pandarus cranchii* in copepodid form. In 1892 Beneden stated that "The *Specilligus curticaudus* of Dana is the male of one of the three *Pandarus* species which he described." [1] Dana did not include *cranchii* in his three species, but did describe *concinnus*, a synonym of *cranchii*, so that Beneden's statement was correct. On the next page of his paper, Beneden described what he called the male of *Pandarus cranchii*, and later the male of *P. affinis*. He said nothing about the size or development of either male, but as they showed no traces of spermatophore receptacles it is evident they were not fully developed. They are both *Specilligus* forms with prominent conspicilla and maxillipeds of the forceps type. The first one may well have been a *cranchii* male in a copepodid stage earlier than Dana's *Specilligus*. It would seem, therefore, as if we might expect the males of other *Pandarus* species to show this same *Specilligus* form during their copepodid stages.

Perissopus communis Rathbun

———. R. Rathbun, Proc. U. S. Nat. Mus., vol. 10, p. 560. pls. 29, 30, 1887. Wilson, Proc. U. S. Nat. Mus., vol. 33, p. 354, pls. 17, 18, 1907.

Occurrence—A single female was obtained from the skin of the caconetta shark, *Carcharhinus limbatus*, which is a new host for this widely distributed species.

CYCLOPODIA

Ergasilus nanus Ed. van Beneden

———. T. & A. Scott, British Parasitic Copepods, vol. 1, 1913, p. 34, pl. 2, figs. 1–5.

Occurrence—This is a typical parasite on the gills of various kinds of mullets, and a dozen females were obtained from the gills of the common striped mullet, *Mugil cephalus*.

Bomolochus attenuatus Wilson

———. Wilson, Proc. U. S. Nat. Mus., vol. 44, p. 198; pl. 21, figs. 28–34, 1913.

Occurrence—This species was originally obtained from the gills of a scorpion fish, *Scorpœna plumieri*, at Montego Bay, Jamaica. A single female was taken here at the Tortugas from the gills of another scorpion fish, *Scorpœna agassizii*, which constitutes a new host as well as locality.

Bomolochus scutigerulus new species

Plate 2, figs. 14 to 22

Occurrence—Four females with egg strings were found on the gills of the red goat fish, *Pseudupeneus maculatus*. These have been given Cat. No. 64036 U. S. Nat. Mus. and become the types of the new species.

The female—Body obovate, broadly rounded anteriorly and strongly narrowed posteriorly (fig. 14). Head fused with the first thoracic segment, one-half wider than long and squarely truncated posteriorly, with sharply rounded corners. The anterior margin of the head is convex with a small three-lobed prominence at the center, which forms the anterior margin of a shield-shaped escutcheon extending onto the dorsal surface of the head. The escutcheon is raised in low relief and shows on either side a dark

[1] Bull. Acad. Roy. Belgique, vol. 33, p. 220.

circular spot, the two looking like a pair of widely separated eyes. The true eyes, however are much farther back in the head and so well hidden beneath the tissues that they are difficult to detect. The second segment is nearly as wide as the head, but the third and fourth segments are strongly narrowed; the fifth segment is narrowed anteriorly and much widened posteriorly through the bases of the fifth legs. The genital segment is quadrangular, nearly twice as wide as long, with angular posterior corners. The abdomen is three-segmented, the segments about the same width but increasing in length posteriorly. The caudal rami are as wide as long, each tipped with three setæ; the egg strings are about as long as the entire body and rather plump.

The first antennæ (fig. 15) are of the usual pattern, the basal segments not much enlarged and completely fused, the three terminal segments rather distinct, diminishing in length and reaching beyond the lateral margins of the head. The fused basal portion bears a row of flattened lamellar setæ along its anterior margin, diminishing in length distally, and ends in a long slender spine posterior to the base of the antepenultimate segment. Interspersed at intervals between the laminate setæ are four long and slender tactile setæ. The second antennæ (fig. 16) are slender, the terminal segment longer than the two basal segments combined, and tipped with a straight spine at the inner corner, three curved claw-like spines and two finger processes at the end. The outer process runs back onto the side of the segment and its outer margin, together with that of the segment itself, is bordered with a narrow corrugated flange. Between the second maxillæ and maxillipeds on the midline is a three-lobed pad (fig. 17); each of its lateral lobes carries a stout process with a rough surface, which probably serves the same purpose as the furca in the Caligidæ. The maxilliped is stout, with a strong terminal claw bent sharply in an S-curve and apparently without setæ. The distal end of the segment to which the claw is attached is considerably wrinkled and furrowed (fig. 18).

The rami of the first legs (fig. 19) are three-segmented, the segments of the exopod almost completely fused but indicated by the outer spines. The second and third exopods (fig. 20, 21) are of the usual pattern, but the endopods are much widened and flattened and are armed with laminate setæ, the middle segment in each endopod with two setæ. The fourth legs are much reduced in size, the endopods like the exopods and both rami with normal setæ. The arrangement of the spines and setæ on these four pairs of legs is as follows: First exopod (one fused segment) 2—6; endopod 0—1, 0—1, 0—5. Second exopod 1—0, 1—1, 3—5; endopod 0—1, 0—2, 0—5. Third exopod 1—0, 1—1, 3—5; endopod 0—1, 0—2, 0—4. Fourth exopod 1—0, 1—1, 3—4; endopod 0—1, 0—0, 0—3. The fifth legs are two-segmented, the terminal segment three times as long as the basal, the former with three setæ at the tip, the latter with one at the anterior distal corner.

Remarks—The curiously modified rostrum in the form of a raised shield extending back onto the dorsal surface of the head is the chief characteristic of this new species and has given rise to the specific name. The absence of setæ on the maxillipeds and the flattened endopods of the second and third legs furnish additional distinctive characters.

TÆNIACANTHODES new genus

Head fused with the first thoracic segment to form an almost spherical cephalothorax; second segment free; third and fourth segments fused into a

trunk; fifth segment free; genital segment enlarged, with two egg strings; abdomen long and narrow; caudal rami elongate and parallel.

First antennæ with fused basal segments bearing a fringe of flattened setæ; second antennæ uncinate; mandibles and maxillæ similar to those of *Tæniacanthus*; maxillipeds of the normal uncinate form. Five pairs of swimming legs; rami of first pair flattened and irregularly fused; rami of second pair three-segmented; exopod of third pair three-segmented, endopod two-segmented; fifth legs uniramose and one-segmented.

Tæniacanthodes gracilis new species

Plate 2, figs. 23-25; Plate 3, figs. 26-29

Occurrence—A single female with egg strings was washed out of the cœlom of a flounder, *Paralichthys squamilentus*. This has been given Cat. No. 64034 U. S. Nat. Mus., and becomes the type of the new genus and species.

The female—Cephalothorax acorn-shaped, slightly longer than wide, with a small knob at each posterior corner (fig. 23). It does not protrude ventrally as in the genus *Tæniacanthus*, but the ventral surface is hollowed like a saucer for the reception of the antennæ, mouth parts and first legs. Second segment much narrower than the cephalothorax and half as long as wide; third and fourth segments fused into an ovate trunk, nearly as wide as the cephalothorax and much longer, narrowed anteriorly and evenly rounded posteriorly. Fifth segment very short, contracted anteriorly and posteriorly and considerably widened centrally through the bases of the fifth legs, but only to half the width of the trunk. Genital segment strongly protruding on either side anteriorly where the egg strings are attached, and contracted posteriorly. Abdomen two-segmented and reaching beyond the tips of the egg strings, the two segments the same width, but the terminal one a half longer than the basal. Caudal rami nearly cylindrical, four times as long as wide, each with three long terminal setæ and a short one near the center of the outer margin. The egg strings are five times as long as wide and in each of them the eggs are arranged in two rows, their outer sides rounded, their inner margins flattened together in a zigzag line.

In the first antennæ (fig. 26) the basal segments are completely fused, leaving only the three terminal ones distinguishable. The proximal end of each antenna is wide and angular and the appendage diminishes regularly in width distally. The anterior margin of the fused basal segments carries a continuous row of flattened setæ, those at the basal corner considerably larger, the others about uniform in size; the terminal segment is tipped with two slender filose setæ. The second antennæ and mouth parts appear to be like those of *Tæniacanthus*, with the exception of the maxilliped which was the only one removed for delineation. It consisted of a moderately stout basal segment and a short and nearly straight terminal claw (fig. 24).

The rami of the first legs (fig. 27) are flattened laminæ, bordered by wide setæ, and are so irregularly fused that the exact structure could not be determined in the single specimen available. Both rami of the second legs are three-segmented (fig. 28) and armed with very long and slender setæ. The spines on the exopod are finger-like with bluntly rounded tips, each carrying a single filose spinule, very similar to those in some *Bomolochus* species. The basal segment of the exopod has no inner seta, the second segment has one and the third segment has five. The endopod has no spines,

the basal segment has one inner seta, the second segment two and the terminal segment four. The basipod has on its outer margin a row of four acuminate spines, and on its inner margin inside the base of the endopod a very short spine and inside of that a second spine nearly as long as the endopod, both spines bluntly rounded at the tip. The exopod of the third leg is three-segmented with 1, 1, and 3 outer spines, 0, 1, and 6 inner setæ. The endopod is two-segmented, the proximal segment unarmed, the distal segment with a finger spine on its outer margin and a very long and stout plumose seta at its tip (fig. 29). The fourth legs were left undisturbed but are much smaller than the first three pairs and are attached to the ventral surface of the fused trunk in front of its center. The fifth legs are uniramose and one-segmented, each a flattened lamina three times as long as wide, with 3 setæ at its tip (fig. 25).

Remarks—The fusion of the third and fourth segments, the free second and fifth segments and the length of the two-segmented abdomen are sufficient both to differentiate and identify this new genus. As the name indicates, it is closely related to *Tœniacanthus*, but the characters just mentioned together with the details of the appendages establish its validity as new.

DICHELESTHIIDÆ

Lernanthropus manicatus new species

Plate 3, figs. 30-36

Occurrence—Two females were found by Dr. Manter on the gills of the saucer-eyed porgy, *Calamus calamus*, and four females from the gills of the jolthead porgy, *C. bajonado*. A single female was taken from the gill rakers of the former host by Dr. Williams. One of the females from the latter host has been made the type of the new species with Cat. No. 64031 U. S. Nat. Mus.

Female—General body form short and plump (fig. 30); cephalothorax ovate, the anterior corners produced into broadly rounded lobes covering the bases of the second antennæ like a pair of large flowing sleeves, whence the specific name. The lateral margins are reentrant at the bases of the lobes and the posterior margin is broadly rounded. The dorsal plate covering the trunk is distinctly divided into two parts, the anterior of which is rectangular and three times as wide as long. Its median third is fused with the dorsal surface of the trunk, while the lateral thirds on either side are free and usually curled down around the sides of the thorax. But they sometimes extend out at right angles to the body axis and on a level with the dorsal surface as shown in the figure. The posterior portion is obovate with very convex sides and truncated ends; it covers the posterior body entirely and most of the fourth legs, leaving only the extreme tips of the endopods and less than half of the exopods visible dorsally.

The genital segment (fig. 31) is enlarged a little anteriorly through the openings of the oviducts, and narrowed a little posteriorly with a minute process at each posterior corner on either side of the abdomen. The latter is one-segmented and longer than wide, with convex lateral margins; the caudal rami are narrow flattened laminæ, five times as long as wide and longer than the gential segment and abdomen combined. They are bluntly rounded at their tips and destitute of setæ.

The first antennæ are rudimentary, consisting of tiny knobs, each tipped with a single filose seta, on the anterior margin of the head between the

bases of the lobes which cover the second antennæ. They are so small as to be readily overlooked and are also easily broken off when the parasite is removed from its host. The second antennæ (fig. 32) have a large and powerful basal segment and a strong curved claw which is armed with a small spine on its inner margin near the base.

The second maxillæ (fig. 33) are moderately stout, the terminal segment laminate and armed with two parallel rows of minute spines, one along the outer margin, the other on the ventral surface. The maxilliped (fig. 34) has a stout basal segment grooved on the inner surface for the reception of the claw, which is stout and curved into a semicircle.

In the first legs (fig. 35) the exopod is a flattened lamina with a rounded tip, armed with six short and blunt teeth extending around the tip and down on the inner margin. The endopod is conical and tipped with a single large spine longer than the endopod itself; inside the base of the endopod is a small spine. In the second legs (fig. 36) the exopod is laminate and considerably enlarged distally where it is tipped with four small spines. The endopod is like that of the first legs, but the terminal spine is much shorter; outside of the exopod is a rounded knob tipped with a slender seta. The third legs are of the usual pattern but quite short and projecting from the ventral surface of the body parallel with each other. The fourth legs have rather narrow rami, the exopods considerably longer than the endopods and projecting for a third of their length behind the dorsal plate, while only the extreme tips of the endopods are visible dorsally. Total length, exclusive of fourth legs, 4 mm. Cephalothorax 1.25 mm. long, the posterior portion 1 mm. wide. Anterior portion of dorsal plate 3 mm. wide, posterior portion 1.87 mm. wide.

Remarks—The distinguishing characters of this new species are the loose sleeves covering the second antennæ and the exceptional width and shape of the anterior portion of the dorsal plate. These two characters can then be supplemented by the structural details of the appendages.

Lernanthropus talipes new species

Plate 3, figs. 37-41

Occurrence—A single female was taken from the gills of the Bermuda chub or rudderfish, *Kyphosus sectatrix*. This is made the type of the new species with Cat. No. 64030 U. S. Nat. Mus.

The female—General form short and stout (fig. 37); cephalothorax narrowed but little anteriorly, with a three-lobed anterior margin, and squarely truncated posteriorly. Anterior portion of dorsal plate nearly as wide as the cephalothorax, with straight and parallel sides. Posterior portion enlarged a little and evenly rounded, reaching just beyond the tips of the caudal rami and covering the basal third of the fourth legs. Genital segment (fig. 38) twice as wide as long, with strongly convex sides; abdomen one-segmented and narrower than the genital segment; caudal rami triangular, swollen at the base and pointed at the tip and without setæ.

First antennæ visible in dorsal view and six-segmented; second antennæ of the usual pattern with the terminal claw slender and strongly curved. Second maxillæ (fig. 39) with a stout basal segment and a moderately slender claw which is jointed at a third of its length from the tip, the basal portion armed with a small spine on the inner margin near the distal end. The maxilliped (fig. 40) has a large basal segment armed on its lateral sur-

face with three diagonal rows of minute spines and a longitudinal row of small processes, each tipped with a minute filose seta. The terminal claw is strong, curved near its tip and armed on its inner margin with three sharp spines.

The first legs (fig. 41) are small and hidden beneath the maxillipeds; the exopod is a flat lamina with five short and blunt spines around the distal end; the endopod is a conical knob tipped with a tiny spine, with a much larger spine inside of its base. The third legs are large and quite divergent, standing out from the lateral margins at right angles to the body axis. The fourth pair are divided to their base (fig. 38), the endopod a narrow and pointed lamina extending straight backward, the exopod also laminate but curved into a semicircle, the convex margin outward, the misshapen and bluntly rounded tip turned inward above the endopod. Total length, exclusive of fourth legs, 3 mm. Cephalothorax 1 mm. long, 1.18 mm. wide.

Remarks—This new species can be recognized at once by the misshapen exopods of the fourth legs, which show plainly in either dorsal or ventral view. The dumb-bell appearance of the cephalothorax and the two portions of the dorsal plate covering the body are also characteristic.

Lernanthropus percis Thomson

———. Thomson, Trans. New Zealand Inst., vol. 22, p. 366, pl. 27, fig. 2, 1889.

Occurrence—Two females were taken from the gills of the spotted jewfish, *Promicrops itaira*, a new host and locality for this parasite. Thomson did not mention the first antennæ, which prove to be six-segmented, with no setæ except two minute ones at the tip of the end segment.

Nemesis atlantica Wilson

———. Wilson, Proc. U. S. Nat. Mus., vol. 60, Art. 5, p. 60, pls. 9 & 10, figs. 61–74, 1922.

Occurrence—Nineteen specimens, including both sexes, were found on the gills of the short-nosed shark, *Hypoprion brevirostris*, and two males on the gills of the Spanish caconetta, *Carcharhinus limbatus*. Both of these sharks are new hosts for the species, but it doubtless infests many of the other large tropical sharks.

Nemesis pallida Wilson

———. Wilson, Bull. 158, U. S. Nat. Mus., p. 464, pl. 33, figs. b–p, 1932.

Occurrence—Seven specimens, including both sexes, were taken from the gills of the short-nosed shark, *Hypoprion brevirostris*, by Dr. Manter. Five other specimens, also including both sexes, were found on the gills of a shark of the same species by Dr. A. S. Pearse. This parasite is also apparently common on the larger tropical sharks, and future investigation will doubtless add to the list of its hosts.

Krøyeria gracilis Wilson

———. Wilson, Bull. 158, U. S. Nat. Mus., p. 453, pl. 29, figs. a–g, 1932.

Occurrence—Ten females were taken from the gills of the sharp-nosed shark, *Scoliodon terræ-novæ*, by Dr. Manter. This is a new host for the species and is the first locality outside of the Woods Hole area where it has been found.

Krøyeria lineata P. J. van Beneden

———. Wilson, Bull. 158, U. S. Nat. Mus., p. 456, pl. 30, figs. j–n, 1932.

Occurrence—Eight females were found by Dr. Manter on the gills of the short-nosed shark, *Hypoprion brevirostris*, and nineteen females on the gills of the caconetta, *Carcharhinus limbatus*, both these sharks being new hosts for the species. Together with the preceding species, therefore, it is likely to be discovered in the future upon the gills of other large sharks which frequent these regions.

Hatschekia linearis Wilson

———. Wilson, Proc. U. S. Nat. Mus., vol. 44, p. 246, pl. 44, figs. 240–246, 1913.

Occurrence—Five females were taken by Dr. Williams from the gills of the hogfish, *Lachnolaimus maximus*, and two females on the gills of the common grunt, *Hæmulon plumieri*. Both hosts as well as the locality are new for the species which has hitherto been confined to the Jamaica coast.

Hatschekia albirubra Wilson

———. Wilson, Proc. U. S. Nat. Mus., vol. 44, p. 239, pl. 41, figs. 209–215, 1913.

Occurrence—A single female was taken by Dr. Williams from the gills of the black angel fish, *Pomacanthus arcuatus*, both the locality and the host being new. These diminutive parasites are usually more or less concealed between the gill filaments and are hence easily overlooked unless the gills are examined under magnification.

Hatschekia uncata Wilson

———. Wilson, Proc. U. S. Nat. Mus., vol. 44, p. 243, pl. 43, figs. 233–239, 1913.

Occurrence—A single undeveloped female from the gills of the crested blenny, *Blennius cristatus*, by Dr. Williams, both the host and the locality being new for the species.

LERNÆIDÆ

Lernæolophus recurvus Wilson

———. Wilson, Proc. U. S. Nat. Mus., vol. 44, p. 252, pl. 46, figs. 256–259, 1913.

Occurrence—A single female was taken from the floor of the mouth of a jolthead porgy, *Calamus bajonado*, by Dr. Manter. This is a new host for the parasite and the present record is the first since the original discovery on the coast of Jamaica.

SPHYRIIDÆ

Pæon ferox Wilson

———. Wilson, Proc. U. S. Nat. Mus., vol. 55, p. 592, pl. 57, figs. 60–66, 1919.

Occurrence—A single female was taken by Dr. Manter from the gill cavity of the small hammerhead shark, *Sphyrna tiburo*, both the host and the locality being new for this species. It seems very probable that the three species of *Pæon* thus far described will prove to be very widely distributed among tropical sharks as examination of the latter progresses.

Rebelula tripartita new species

Plate 6, figs. 72-75

Occurrence—A single female was taken from the flesh of the jolt-head porgy, *Calamus bajonado,* by Dr. Williams. It has been given Cat. No. 59768 U. S. Nat. Mus. and becomes the type of the new species.

Female—Cephalothorax narrow and five or six times as long as wide, soft, transversely wrinkled, and passing without change of diameter into the smooth chitinous neck. Head separated from the rest of the cephalothorax by a deep groove, about as long as wide and much narrower than the inflated collar into which it sets. The transverse wrinkles begin immediately behind the collar and extend halfway to the horns. This wrinkled portion is soft, pliable and a little wider than the smooth, chitinous portion which follows. The neck is bent ventrally where it joins the trunk, but otherwise is almost straight and is neither twisted nor enlarged in the region of the horns. The latter are attached one-third of the distance between the head and trunk and are three in number, one dorsal and two latero-ventral. Each consists of a short basal portion which divides distally into three branches, shorter than the basal portion, conical and blunt, and some of them armed with small knobs.

The trunk is obcordate and almost as thick as wide, with smooth and rounded posterior corners, and with four pits on either side of both the dorsal and ventral surfaces. The genital processes are small circular disks, flattened against the ventral surface of the trunk near its posterior margin. The abdomen is represented by a median posterior lobe, fused with the trunk and carrying a stout posterior process attached to its dorso-lateral margin on either side. These processes are divergent and covered with respiratory cylinders, the distal ones considerably longer than the proximal.

Remarks—This female is the same size as *Rebelula gracilis* and has similar body proportions and other resemblances. But at the same time there are such important differences in the structure of the cephalothorax, the neck and the horns as to make the identity of the two impossible. The males in this genus add greatly to specific recognition and when they are found it will be quite easy to complete the specific distinction.

LERNÆOPODIDÆ

Brachiella exigua Brian

———. Brian, Copepodi parassiti dei Pesci d'Italia, p. 104, pl. 7, fig. 1, 1906.

Occurrence—A single female was taken from the gills of the silver hake, *Merluccius bilinearis,* which is a new host. This is the first time also that the species has been found outside of the Mediterranean, and adds another parasite to the long list already known to infest this fish.

Brachiella appendiculata (Krøyer)

Anchorella. Krøyer, Naturhistorisk Tiddskrift, vol. 3, p. 305, pl. 16, fig. 7, 1863.

Occurrence—Two females were taken by Dr. Williams from the mouth of a gurnard, *Bellator militaris,* and another female from the gills of the jolt-head porgy, *Calamus bajonado.* Krøyer's specimens came from Valparaiso and all of those since found have come from European seas, so that this is the first to be found in North American waters on either side of the continent.

Clavella inversa Wilson

———. Wilson, Proc. U. S. Nat. Mus., vol. 44, p. 261, pl. 50, figs. 275–281, 1913.

Occurrence—A single female with egg strings was found by Dr. Manter on the gills of the jolt-head porgy, *Calamus bajonado,* and two females by Dr. Williams upon the gills of another fish of the same species, which is a new host.

Charopinus quaternius new species

Plate 4, figs. 42-49

Occurrence—Six females and a male were found on the gills of a slender gurnard, *Peristedion gracilis,* and a single female on the gills of the dorado, *Coryphœna hippurus.* One of the females has been selected as the type of the new species with Cat. No. 64010 U. S. Nat. Mus.; the others become paratypes with Cat. No. 64011 U. S. Nat. Mus.

Female—Cephalothorax broad, elongate and in line with the trunk (fig. 42); head with a dorsal carapace which covers the entire dorsal surface and part of the lateral surfaces. Neck long and cylindrical with indistinct transverse grooves; trunk elongate, flattened dorso-ventrally for its entire length and rounded posteriorly. No separate abdomen, genital process or caudal rami. Two pairs of posterior processes (fig. 43), one median and one lateral, very unequal in length and both pairs ventral to the egg strings. The median pair are flattened dorso-ventrally, narrow lanceolate in form and sharply pointed. They are five-sevenths as long as the trunk and their bases are fused on the ventral midline. The lateral pair are also flattened laminæ triangular in shape, one at each posterior corner of the trunk, and are one-fourth as long as the median pair. The egg strings are each attached to the posterior end of the trunk between the median and lateral process and dorsal to both, and are as long as the entire body.

The first antennæ (fig. 44) are two-segmented, the basal segment considerably swollen with a single spine at the outer distal corner, the terminal segment narrower, conical and tipped with one spine. The second antennæ (fig. 45) are turned across the frontal margin of the head and meet on the midline; each is biramose with a stout unsegmented exopod and a shorter two-segmented endopod tipped with a minute knob. The mouth tube (fig. 46) is short and wider than long; each first maxilla is without any discernible palp and three-segmented, the segments diminishing in size distally. The second segment is armed with a spine at the inner distal corner and the third segment with one at its tip. The second maxillæ are removed some distance behind the maxillipeds and are entirely separate except a slight adhesion of the inner surfaces of the two bullas. Each is cylindrical, short, curved like a parenthesis mark and tipped with a cup-shaped bulla. The inner surfaces of the two bullæ are in contact and slightly fused, but each is fastened independently to the host. The maxilliped (fig. 47) is close behind the mouth tube; it has a long and stout basal segment which is grooved on its inner surface distally for the reception of the second segment and claw. The second segment is less than half the length of the first and its tip projects as a rounded process inside the base of the claw, which is short and slender.

Male—At first sight the male (fig. 48) seems to resemble those of the genus *Lernœopodina,* but more careful examination reveals the characters of *Charopinus* males. The dorsal portion of the cephalothorax projects far forward in a large rounded knob, and the mouth tube is attached to the

ventral surface at right angles to the body axis. The second maxillæ and maxillipeds are so close to the mouth tube that the former are in actual contact with it. The first two thoracic segments carry rudimentary legs and the abdomen has a pair of slender and curved caudal rami. The second maxillæ (fig. 49) are the attachment appendages and are clasped around one of the median posterior processes of the female. Both these second maxillæ and the maxillipeds are exceptionally large and fasten the male so securely that detachment without injury to the appendages is very difficult. Total length 0.35 mm.

Remarks—Both sexes of this species exhibit distinctive characters; in the female the cephalothorax is not turned back against the trunk, and there are four posterior processes, both pairs ventral to the egg strings. In the male the cephalothorax has a large anterior projection, the appendages are crowded forward against the mouth tube and there are two pairs of two-segmented legs.

Quaternius, having four parts, alluding to the posterior processes.

Thysanote pomacanthi Krøyer

———. Krøyer, Naturhist. Tidsskrift, ser. 3, vol. 2, p. 288, pl. 15, fig. 1, a–i, 1863.

Occurrence—A single female was taken from the gill cavity of a black angel fish, *Pomacanthus arcuatus*, the same host from which the original type specimens were obtained.

Naobranchia lizæ (Krøyer)

Anchorella. Krøyer, Naturhist. Tidsskrift, ser. 3, vol. 2, p. 296, pl. 16, 1863.

Occurrence—A single female was found on the gills of the short-nosed batfish, *Ogcocephalus cubifrons*, which is a new host for the parasite.

CHONDRACANTHIDÆ

Chondracanthopsis nodosus (Müller)

Lernæa. Müller, Zoologiæ Danicæ Prodromus, p. 227, No. 2749, 1776. *Chondracanthopis*, Wilson, Bull. 158, U. S. Nat. Mus. p. 508, pl. 39, figs. a–f, 1932.

Occurrence—A single juvenile female was obtained from the gills of the green parrot-fish, *Sparisoma viride*. This specimen was less than half the length of the mature female, but the distal segments of the legs showed distinctly the characteristic boot shape of the genus.

Pseudochondracanthus hexaceraus new species

Plate 4, figs. 50-57

Occurrence—Four females, two of them with egg strings and two with attached males, were taken from the gills of the southern puffer, *Spheroides spengleri*. One of these was dissected for the appendages and the other three were made types of the new species, with Cat. No. 64014 U. S. Nat. Mus.

Female—Body short and stout (fig. 50); head as wide anteriorly as long but somewhat narrowed posteriorly; the anterior margin convex with two rounded knobs at the center, separated by a V-shaped sinus. The anterior corners are broadly rounded and the lateral and posterior margins nearly straight. No dorsal carapace is visible but the dorsal surface at the center

of the posterior margin is raised into a rounded knob (fig. 54). The single free segment is very short and nearly as wide as the posterior margin of the head and carries a pair of rudimentary legs. The remaining thoracic segments are fused into a trunk a trifle wider than long, abruptly narrowed at the anterior end where it joins the free segment and produced into broadly rounded lobes at the posterior corners. The posterior margin between the lobes is convex and to it is attached the genital segment, which is narrowed anteriorly and widened posteriorly, with the egg strings at its posterior corners. The abdomen is incompletely separated from the genital segment, very short, one-segmented, and carries a pair of circular unarmed caudal rami. The egg strings are stout and two-thirds as long as the entire body.

The first antennæ (fig. 51) are pear-shaped, the basal segment much swollen and projecting considerably in front of the anterior margin of the head. The terminal portion is four-segmented, the segments diminishing in size outwardly, the last two with setæ. The second antennæ (fig. 52) have a short basal segment and a long sickle-shaped terminal claw. They are attached to a transverse chitin rib which runs across the bases of the two horns which project from the anterior corners of the head. There are two other pairs of chitinous horns along the sides of the head; the middle pair is very large with a wide base and a rounded tip, and like the anterior pair each horn is a flattened lamina. The horns of the posterior pair are very small, narrowed and pointed at the tip and cylindrical rather than laminate.

The mouth is at the posterior margin of the head, and the sides of the head opposite the mouth are not swollen as in *diceraus*. Of the mouth parts (fig. 53) the mandibles are nearly straight and toothed along both margins. The first maxillæ are also toothed along both margins and are without a spur. The second maxillæ are enlarged and turned backward inside the bases of the two posterior pairs of horns, and then inward toward the midline. The terminal segment of these maxillæ carries a seta on its inner margin and ends in a long curved point, but there is no claw. One of the rudimentary legs on the free segment is shown in figure 57; the ramus is one-jointed and armed with four minute spines. Total length of female 1.30 mm. Width of head 0.56 mm., of trunk 0.66 mm.

The Male—The male (fig. 55) is a pygmy attached to the genital segment of the female. The cephalothorax is larger than the rest of the body and is covered with a carapace; the rest of the body is indistinctly segmented with no distinction of parts and ends in a pair of sharply pointed caudal rami. The second antennæ are attached close to the frontal margin and end in a pair of sickle-shaped claws. The mouth tube is at right angles to the body axis and carries on its sides the first maxillæ. The second maxillæ and maxillipeds are a short distance behind the mouth; the maxillipeds have a swollen basal segment and a short semicircular claw and look like figure 56 when viewed from the side. There are no rudimentary legs. Total length of male 0.45 mm.

Remarks—This species can be readily recognized by the three pairs of horns on the lateral margins of the head and the relative proportions of the head and trunk.

Hexaceraus, six-horned.

PHARODES new genus

Head fused with the first segment to form a triangular cephalothorax, with the base of the triangle anterior and with large lateral expansions.

The rest of the thorax and the genital segment fused into a trunk which is produced laterally on either side into a large nodular process, curved backward along the side of the abdomen. Dorsal surface of both trunk and expansions with flattened knobs. Abdomen an unpaired median process extending posteriorly between the lateral expansions of the trunk, separated from the latter on the ventral surface but not at all on the dorsal surface, where it is covered with two tripartite plates, one behind the other. Two pairs of antennæ, the second pair uncinate; first maxillæ styliform, second pair uncinate; three pairs of leg rudiments. Pharos, a wide cloak without sleeves.

Pharodes tortugensis new species

Plate 6, figs. 76-82

Occurrence—Single females were obtained by Dr. Williams from the gills of two gobies, *Bathygobius soporator*, three blennies, *Blennius cristatus*, and one jolthead, *Calamus bajonado*. One of those from a blenny has been made the type for the new genus and species, with Cat. No. 59767, U. S. Nat. Mus.

Female—Head nearly twice as wide as long, the lateral expansions broadly rounded, both pairs of antennæ visible dorsally in front of the anterior margin. The mouth and mouth parts on the ventral surface are some distance behind the anterior margin. There is no neck between the cephalothorax and trunk and in the latter there are no traces of segmentation. Each lateral expansion covers the entire length of the side of the trunk from the anterior margin of the second segment to the posterior margin of the genital segment. The process is wider and thicker and more than twice the size of the trunk itself and reaches back to the posterior end of the abdomen. Its dorsal surface is raised slightly in three flattened knobs, and on the ventral surface directly under the anterior dorsal knob is a branch process which extends downward and backward and may well represent one of a third pair of legs. Between the lateral expansion and the trunk is a shallow groove and between the two grooves the dorsal surface of the trunk is somewhat raised and shows a rounded knob at its posterior end over the genital segment.

The abdomen is as long as the trunk, one-segmented and tongue-shaped, wider in front than behind. Ventrally there are no traces of division, but dorsally it carries two tripartite knobs as though it might be made up of two segments fused. There are no caudal rami. The first antenna is five-segmented, the basal segment the longest, and is very meagerly armed with setæ. The second antenna has a large terminal claw which projects in front of the anterior margin of the head and evidently serves as the chief organ of attachment. The first maxillæ are long and stylet-shaped, with a rather blunt tip; the second maxillæ are three-segmented with a short end claw, abruptly bent near its base. There are two pairs of leg rudiments on the ventral midline, one pair on the first segment just behind the mouth parts and the second pair behind these on the second segment. Each leg of these two pairs is a single small knob without segmentation or armature. The ventral branches of the lateral thoracic processes may well represent a third pair of legs, larger and longer than the others, and somewhat removed from the midline. The ovisacs are larger than the lateral thoracic processes and the eggs are exceedingly small, the number in each sack mounting well into the thousands. Total length of female 1.54 mm. Greatest width including the lateral processes 2 mm. Length of ovisacs 1.60 mm.

Remarks—This new copepod seems fairly common and not confined to a single species of fish. The antennæ and mouth parts, the lateral processes of the thorax and the dorsal knobs and projections, the leg rudiments and the multiseriate ovisacs all indicate a close affinity with the Chondracanthidæ, but at the same time the general makeup is so different from that of other members of this family as to constitute a new genus.

Acanthochondria crassicornis (Krøyer)

Chondracanthus. Krøyer, Naturhistorisk Tidsskrift, vol. 1, p. 203, pl. 2, fig. 10, 1835.

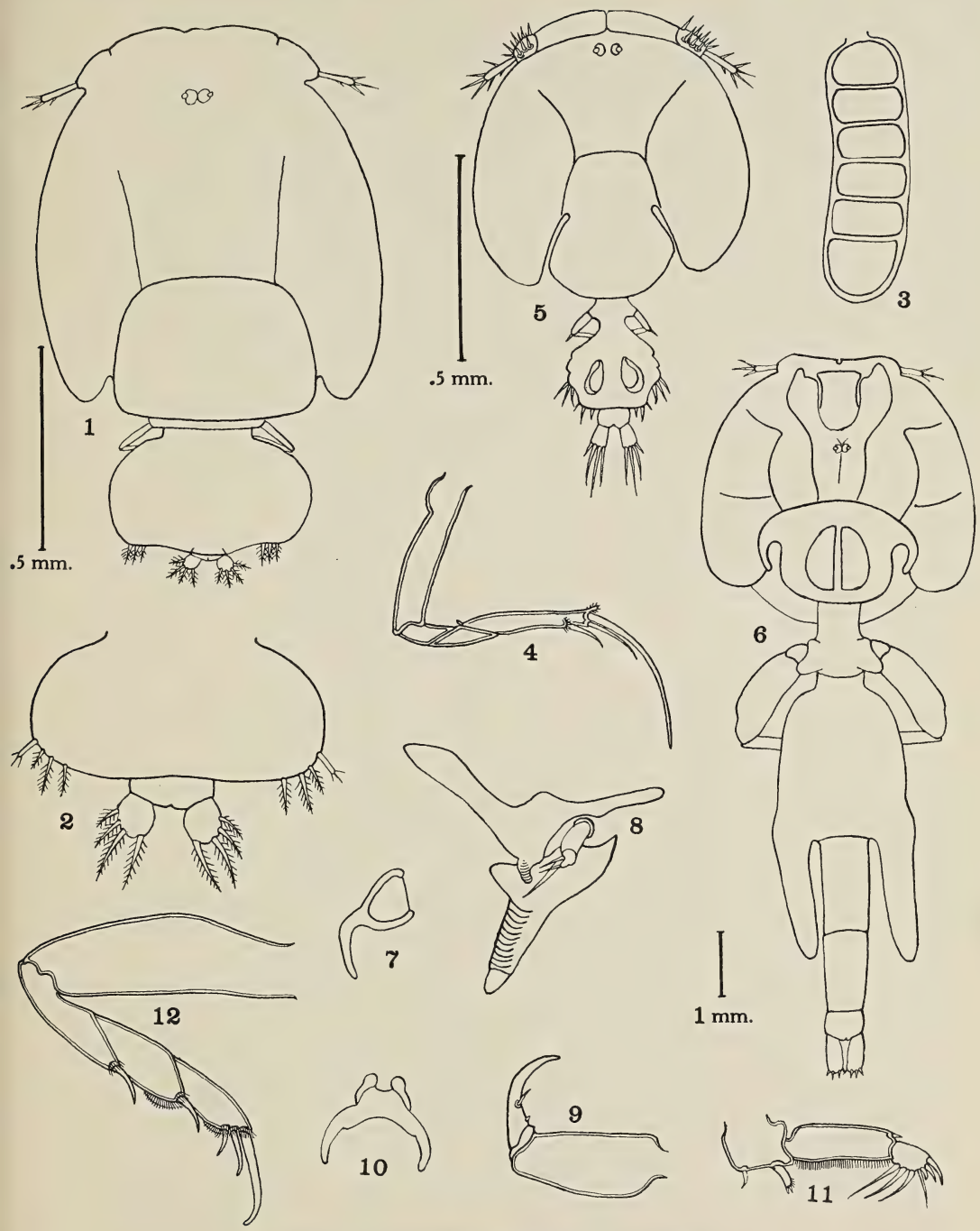
Occurrence—A single female was taken from the gills of the petite jaquette, *Eupomacentrus planifrons*, at the Tortugas Laboratory. This is a new host and is the first record of the species from American waters, Atlantic or Pacific.

HARPACTICIDÆ

Cancrincola jamaicensis Wilson

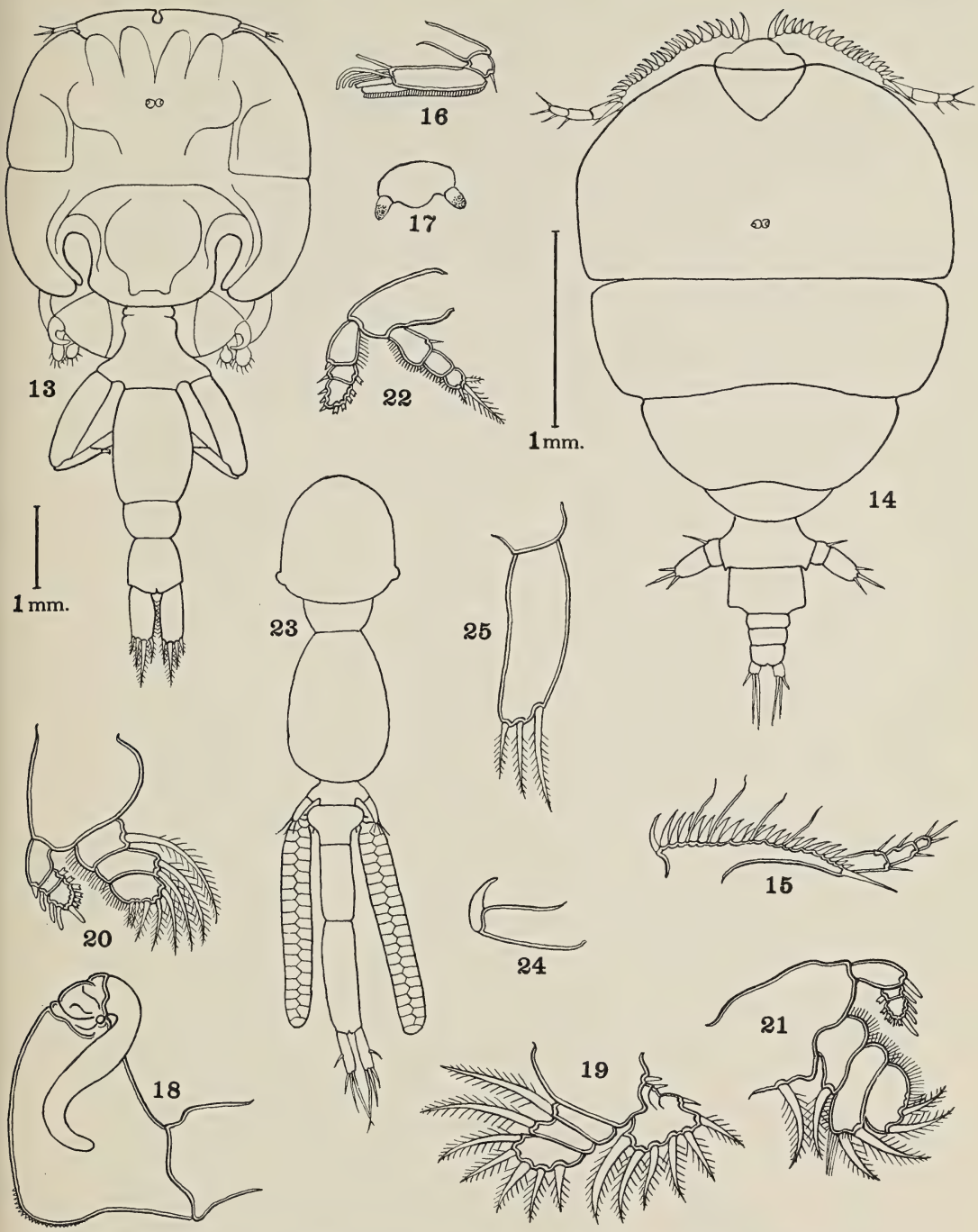
———. Wilson, Proc. U. S. Nat. Mus., vol. 44, p. 264, pl. 52, figs. 289–302, 1913.

Occurrence—A few specimens were taken by Dr. A. S. Pearse from the branchial cavity of *Microphrys bicornutus*, which is a new host as well as locality for this parasitic Harpactid.



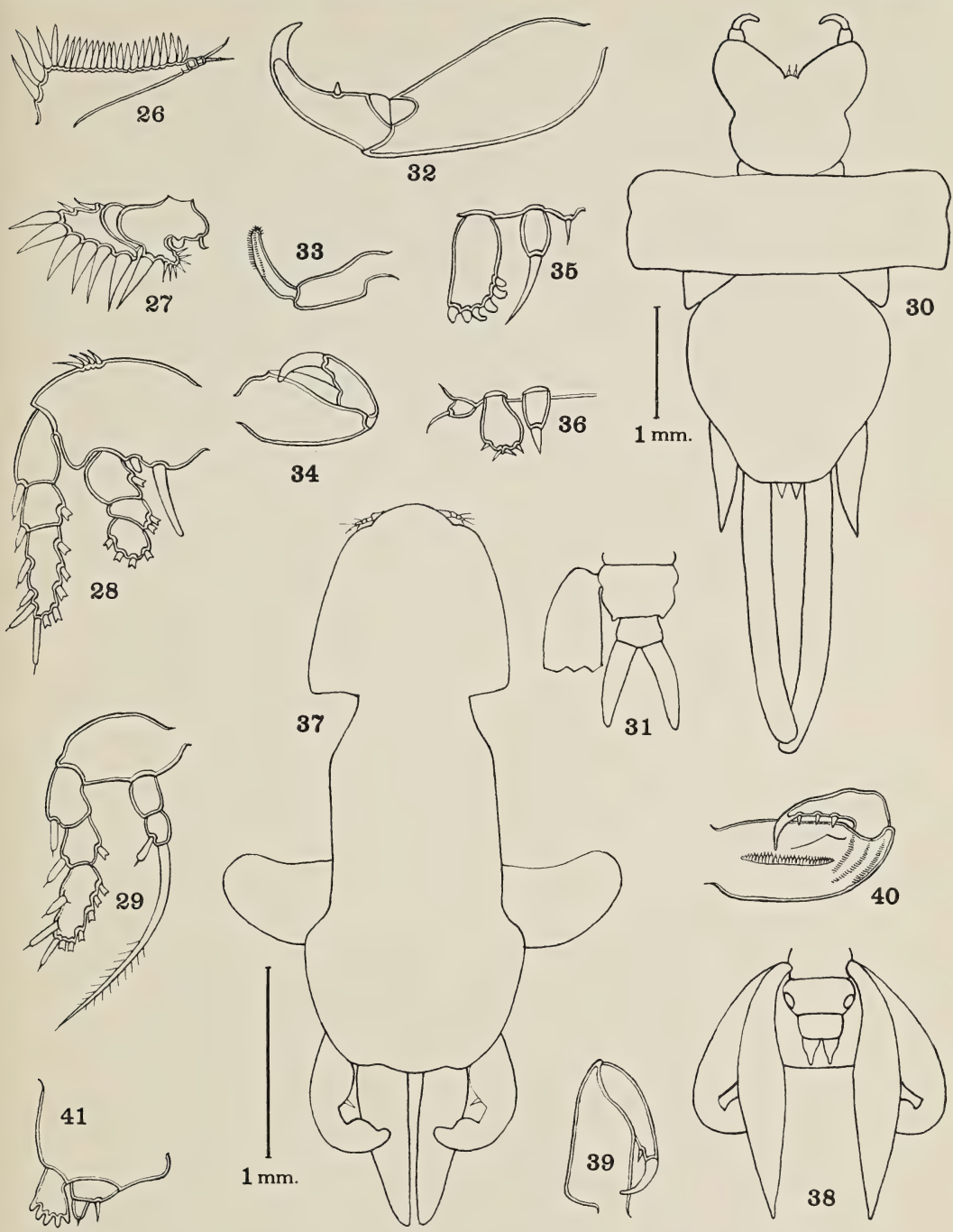
Anuretes parvulus and *Paralebion elongatus.*

Fig. 1, *Anuretes parvulus*, dorsal view of mature female. Fig. 2, Genital segment and abdomen of immature female. Fig. 3, An egg string. Fig. 4, Fourth leg. Fig. 5, Dorsal view of male. Fig. 6, *Paralebion elongatus*, dorsal view of female. Fig. 7, Maxillary hook. Fig. 8, First maxilla. Fig. 9, Maxilliped. Fig. 10, Furca of male. Fig. 11, First swimming leg. Fig. 12, Fourth leg.



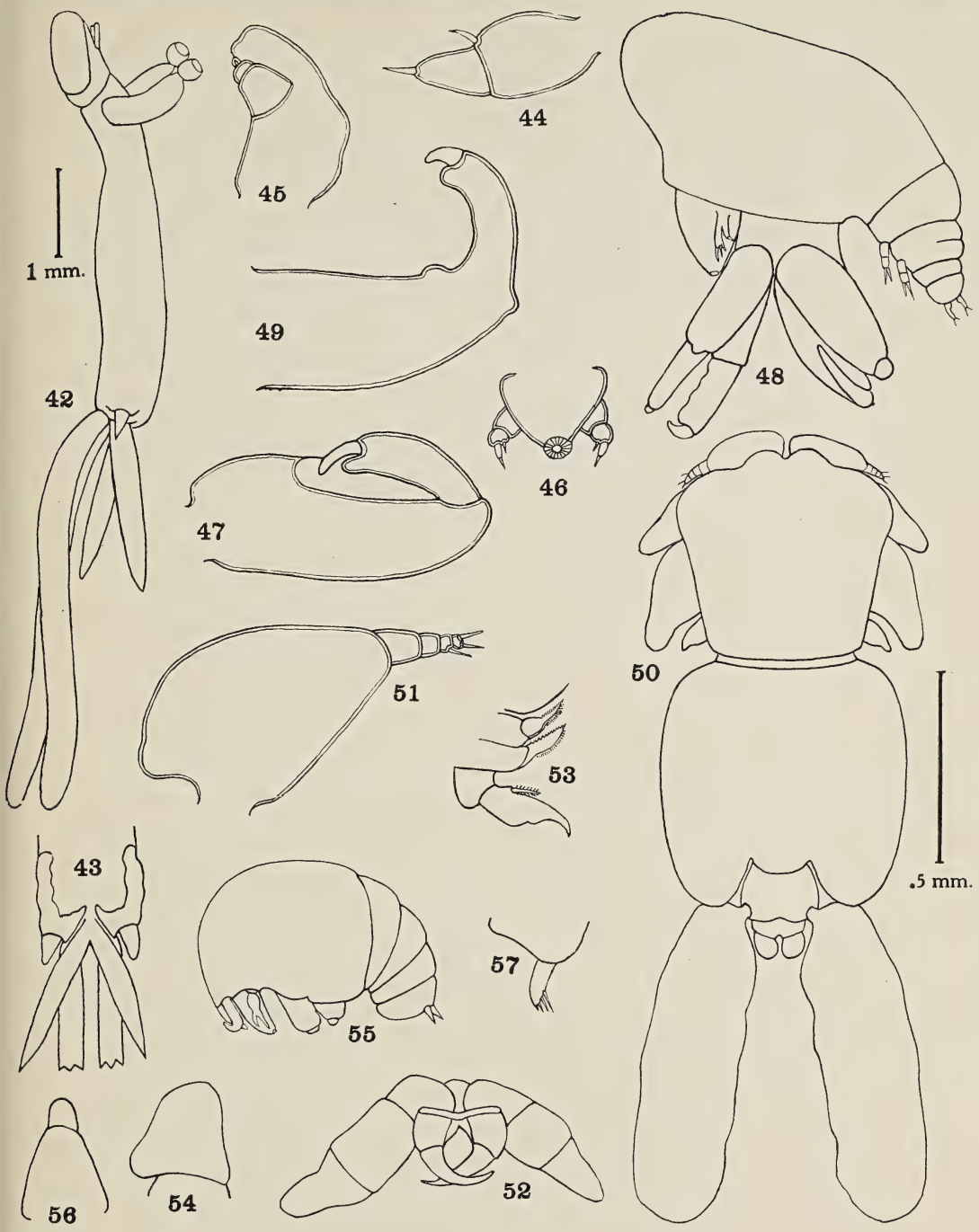
Paralebion elongatus, Bomolochus scutigerulus and *Tæniacanthodes gracilis.*

Fig. 13, *Paralebion elongatus*, dorsal view of male. Fig. 14, *Bomolochus scutigerulus*, dorsal view of female. Fig. 15, First antenna. Fig. 16, second antenna. Fig. 17, Attachment pad. Fig. 18, Maxilliped. Fig. 19, First swimming leg. Fig. 20, Second leg. Fig. 21, Third leg. Fig. 22, Fourth leg. Fig. 23, *Tæniacanthodes gracilis*, dorsal view of female. Fig. 24, Maxilliped. Fig. 25, Fifth leg.



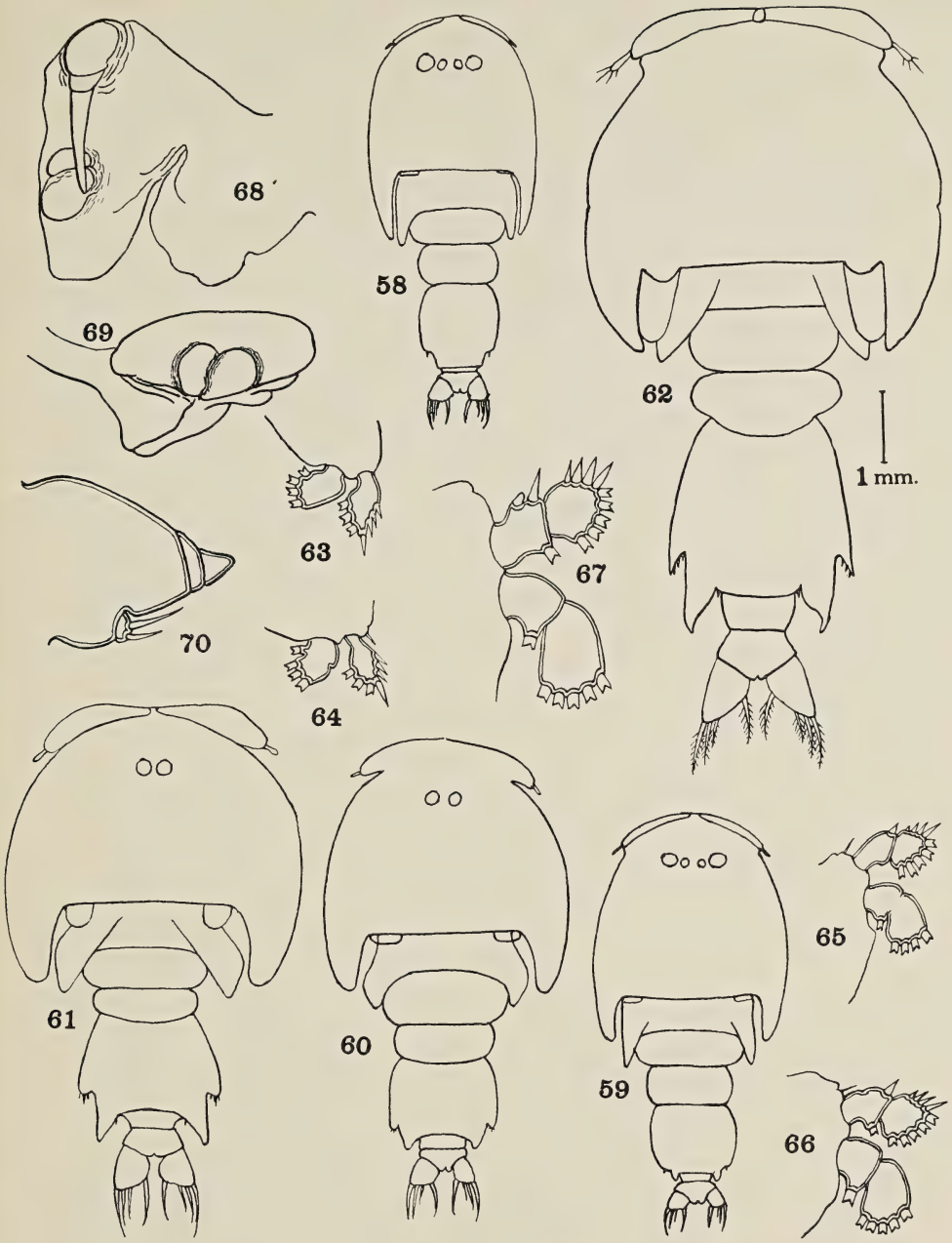
Tæniacanthodes gracilis, Lernanthropus manicatus, and *Lernanthropus talipes*

Fig. 26, *Tæniacanthodes gracilis,* first antenna. Fig. 27, First swimming leg. Fig. 28, Second leg. Fig. 29, Third leg. Fig. 30, *Lernanthropus manicatus,* dorsal view of female. Fig. 31, Ventral view of genital segment and abdomen. Fig 32, Second antenna. Fig. 33, Second maxilla. Fig. 34, Maxilliped. Fig. 35, First swimming leg. Fig. 36, Second leg. Fig. 37, *Lernanthropus talipes,* dorsal view of female. Fig. 38, Ventral view of fourth legs, genital segment and abdomen. Fig. 39, Second maxilla. Fig. 40, Maxilliped. Fig. 41, First leg.



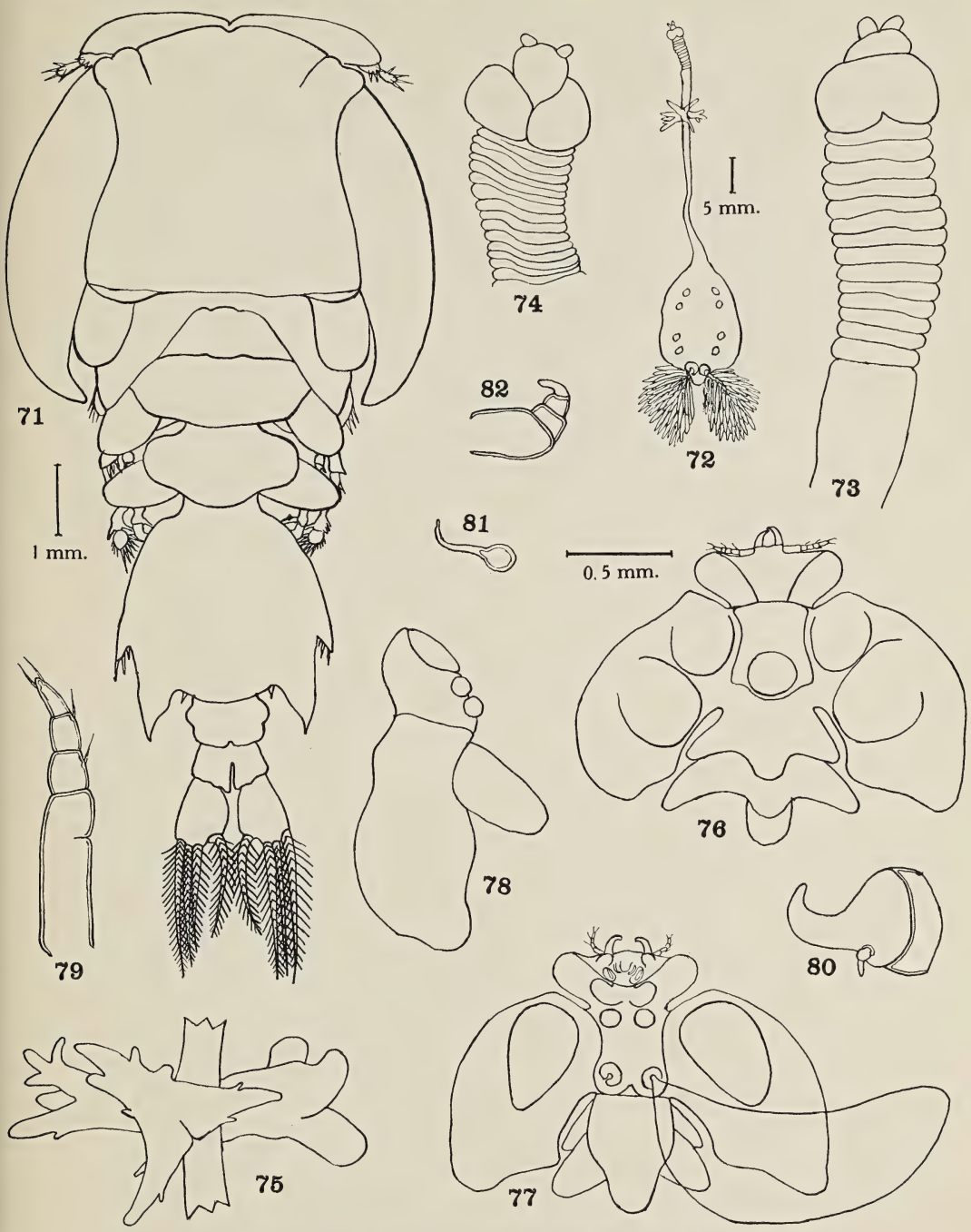
Charopinus quaternius and *Pseudochondracanthus hexaceraus.*

Fig. 42, *Charopinus quaternius*, side view of female. Fig. 43, Ventral view of posterior end of trunk, showing posterior processes and egg strings. Fig. 44, First antenna. Fig. 45, Second antenna. Fig. 46, Mouth tube and first maxillæ. Fig. 47, Maxilliped. Fig. 48, Side view of male. Fig. 49, Second maxilla, male. Fig. 50, *Pseudochondracanthus haxaceraus*, dorsal view of female. Fig. 51, First antenna. Fig. 52, Second antennæ. Fig. 53, Mandible, first and second maxilla. Fig. 54, Side view of head, showing dorsal protuberance. Fig. 55, Side view of male. Fig. 56, Maxilliped of male in side view. Fig. 57, Rudiment of first leg of female.



Pandarus cranchii.

Fig. 58, Dorsal view of male, fifth copepodid stage. Fig. 59, Dorsal view of male, sixth copepodid stage. Figs. 60 to 62, Dorsal view of male, first three adult stages. Figs. 63 to 66, Fourth legs of above males in the same order and magnified alike. Fig. 68, Maxilliped of fully grown male (12 mm. long). Fig. 69, Maxilliped of male in copepodid stage. Fig. 70, First maxilla of male in copepodid stage.



Pandarus cranchii, Rebelula tripartita and *Pharodes tortugensis*

Fig. 71. Dorsal view of *Pandarus cranchii* male, fully developed. Fig. 72, Ventral view of *Rebelula tripartita* female. Fig. 73, Dorsal view of head and neck, enlarged. Fig. 74, Ventral view of same. Fig. 75, Enlarged view of horns on the neck. Fig. 76, Dorsal view of *Pharodes totugensis* female. Fig. 77, Ventral view of same, with outline of one ovisac. Fig. 78, Side view of same, showing ventral branch of lateral process (third leg?). Fig. 79, First antenna. Fig. 80, Second antenna. Fig. 81, First maxilla. Fig. 82, Second maxilla.

PAPERS FROM TORTUGAS LABORATORY, VOL. XXIX

XIII

THE WATER EXCHANGES OF LIVING CELLS

III: The Application of a Photographic Method to the Determination of the Non-Solvent Volume of the Eggs of Echinometra lucunter

By James L. Leitch
University of California

With one plate and one graph

[Issued March 10, 1936.]

THE WATER EXCHANGES OF LIVING CELLS

III: The Application of a Photographic Method to the Determination of the Non-Solvent Volume of the Eggs of Echinometra lucunter

INTRODUCTION

Photography has been applied to the study of living cells and tissues by such workers as Canti (1928) on tissue cultures, Lewis and Gregory (1929) on the development of rabbit eggs, and Ponder (1930) on the measurement of erythrocytes. In an earlier publication, Leitch (1934) raised the question of the feasibility of a photographic method in the study of the osmotic behavior of cells. The present paper outlines such a method and discusses some of the factors involved in the study of the water exchanges of the eggs of the sea-urchin, *Echinometra lucunter*, using measurements of photographs of eggs at equilibrium in dilute sea-water solutions.

The writer wishes to thank the Carnegie Institution of Washington for making this investigation possible and Dr. W. H. Longley, Director of the Tortugas Laboratory, for his very helpful advice. Grateful acknowledgment is made to Professor S. C. Brooks of the University of California for his critical reading of the manuscript.

MATERIALS AND METHODS

The eggs of *Echinometra lucunter* were obtained and treated in the manner previously described (Leitch, 1931, 1934). The procedure for the determination of equilibrium conditions was that employed by Leitch (1934).

A simple camera box was constructed and supported in place directly above the ocular of a monocular microscope. A well-braced upright of wood from a large metal base served as the support and permitted vertical adjustment of the position of the camera. A 250 watt, 32 volt, condensed-filament bulb served as a source of light and was placed beneath the metal base in such a manner that the filament was in the optical axis of the microscope. As a precaution against heating effects, the watch-glass containing the eggs was supported in a finger bowl of water in such a way that there was a one-inch layer of water between the two. The image of the eggs was focused on a ground-glass plate, after which the plate holder containing Eastman, 3¼ x 4¼ D.C. Ortho plates was slipped into position and the exposure made. Less than 10 seconds of total illumination was needed for the entire process of focusing and exposing, and the change in temperature of the sea-water containing the eggs during the duration of the experiment was less than 0.5°C.

Measurements of plates and prints were made by means of a pair of straight, sliding calipers, which could be read accurately to tenths of millimeters by means of a vernier scale. All values used in the calculations are from measurements of the prints, since measurements on the negatives could only be made accurately by placing the jaws of the calipers in contact with the gelatin surface, a procedure which marred it and rendered the negatives unsuited for further work. Five measurements were made on different diameters of each egg so that any variations in the sphericity of the eggs could be ascertained. The mean and the maximum deviations of each group of five measurements were noted.

EXPERIMENTS

PRELIMINARY EXPERIMENTS

Before proceeding to actual experiments on the sea-urchin eggs, the microscope and camera were calibrated to determine the variation in magnification in various regions of the photographed field. It was immediately found necessary to cut down the field photographed until its diameter was slightly less than the longer dimension of the plates. In Plate I, figures 1

TABLE 1—*Measurements of 100 micra divisions of a stage micrometer photographed using a 6xb ocular and a No. 6 objective.*

Region of field	Measurements Unit=0.1 mm.					Average Meas.	Calibration 1 div.=u
0.4 to 0.5	190	190	191	191	190	190.4	0.525
0.5 to 0.6	188	188	187	187	188	187.6	0.530
0.6 to 0.7	185	186	186	185	185	185.4	0.539
0.7 to 0.8	187	186	186	187	186	186.4	0.537
0.8 to 0.9	189	189	189	189	188	188.8	0.530
							Mean=0.532

and 2, and tables 1 and 2 are given the photographs and measurements, respectively, of calibration experiments employing different Leitz optical systems. The results (table 1) using a 6xb ocular and a No. 6 objective show a variation in magnification from the mean of approximately 2 per cent, while for a combination of a 6xb ocular and a No. 5 objective (table 2) the variation is less than 1 per cent. All the eggs photographed by the latter optical system may be considered and their volumes calculated, using the mean calibration value for the entire plate. This introduces an error of not more than 3 per cent in the calculated volumes. A third combination of a 6xb ocular and a No. 3 objective was tried and, although more eggs were included in the photographed field, this advantage was offset by the greater difficulty of obtaining accurate measurements of the smaller images.

The maximum variation in the measurements with the calipers was ±0.1 mm. This represents the accuracy of the method of measuring. In table 3 are given the measurements and calculations for the eggs in one photograph. The majority of the eggs show a maximum deviation either

TABLE 2—*Measurements of 100 micra divisions of a stage micrometer photographed using a 6xb ocular and a No. 5 objective.*

Region of field	Measurements Unit=0.1 mm.					Average Meas.	Calibration 1 div.=u
1.4 to 1.5	169	169	170	170	169	169.4	0.590
1.5 to 1.6	168	168	168	168	168	168.0	0.595
1.6 to 1.7	167	166	168	168	167	167.2	0.598
1.7 to 1.8	167	168	167	167	168	167.4	0.597
1.8 to 1.9	169	169	169	170	169	169.2	0.591
							Mean=0.594

equal to or less than the error of measurement. However, in practically every print, one or more eggs showed a maximum deviation somewhat greater than this (See No. 1 of table 3) indicating that such eggs were not perfectly spherical. Therefore, in all calculations of the mean volume of the eggs in a photograph, any egg showing a maximum deviation of more than 0.1 mm. was excluded.

TABLE 3—*Measurements of diameters and calculated volumes of eggs in normal sea-water.*

No.	Measurements Unit=0.1 mm.					Average Diam.	Max. Dev.	Diam. Microns	Volume $x10^2u^3$
1	185	185	181	182	186	183.8	2.8	...	....
2	186	187	187	188	187	187.0	1.0	97.1	4794
3	181	180	182	181	181	181.0	1.0	94.0	4349
4	181	181	182	182	182	181.6	0.6	94.3	4391
5	180	181	179	179	181	180.0	1.0	93.5	4280
6	182	182	183	183	183	182.6	0.6	94.8	4461
7	185	183	184	184	184	184.0	1.0	95.5	4560
8	185	184	185	184	185	184.6	0.6	95.8	4604
									Mean=4491±41

EXPERIMENTS ON THE EGGS OF ECHINOMETRA LUCUNTER

The values for the non-solvent volume of the eggs of this sea-urchin, as summarized in table 4, were determined by three different procedures, namely: (1) filar micrometer measurements of groups of fifty eggs after they had been in diluted sea-water solutions at least one hour (abbreviated

in the table to "Equilibrium, ocular"), (2) measurements of photographs of groups of eggs at equilibrium (abbreviated in the table to "Equilibrium, photographic"), and (3) filar micrometer measurements of single, isolated eggs followed during the swelling process for approximately 2½ hours. In all cases, the eggs, after being exposed to the experimental solutions, were returned to normal sea-water, fertilized and their development observed. This was done to ascertain whether or not the experimental procedure had irreversibly injured the eggs. The non-solvent volume of the eggs was calculated by using the following equation developed by McCutcheon, Lucké, and Hartline (1931):

$$P_o\ (V_o - b) = P_{ex}\ (V_{ex} - b)$$

where V_o, V_{ex}, and P_o, P_{ex} are the initial and final egg volumes and internal osmotic pressures, respectively, and b is the non-solvent volume.

DISCUSSION

The preliminary experiments definitely show that the photographic method can be used as a means of studying the water exchanges of cells.

The probable error of the mean volume calculated from any one photograph never exceeds 2 per cent of the mean (in table 3, it is 0.9 per cent). For the mean volume of groups of ten eggs calculated from measurements made with a filar micrometer, the probable error never exceeds 1.5 per cent of the mean. The two methods can therefore be said to be subject to an error of the same order of magnitude when one is considering the average volume of a group of eggs not undergoing swelling. It should, however, be realized that in using the filar micrometer method on swelling eggs, the probable error would be much larger because of volume changes occurring during the time required for making measurements of ten or more eggs. This error may be calculated to reach 5 or 10 per cent, depending on the conditions, being greater in the more dilute solutions and at the beginning of the swelling process. A further advantage of the photographic method is that a greater number of experiments can be carried to completion with a single group of eggs. With ocular methods an entire day is necessary to accumulate data sufficient for use in calculating the non-solvent volume or the rate of swelling of but one group of eggs. An error is thus introduced since the eggs used during the later part of the experiment have been standing in sea-water for several hours, during which time their permeability characteristics may have been altered. In contrast to this, by using photography triplicate experiments can be performed on the same group of eggs within 2 or 3 hours, so that all eggs are in approximately the same condition.

The final test of the applicability of the photographic method is afforded by comparison of the values for the non-solvent volume of the eggs obtained by this method with those determined by direct visual observation. In table 4 are summarized the results of several experiments on the non-

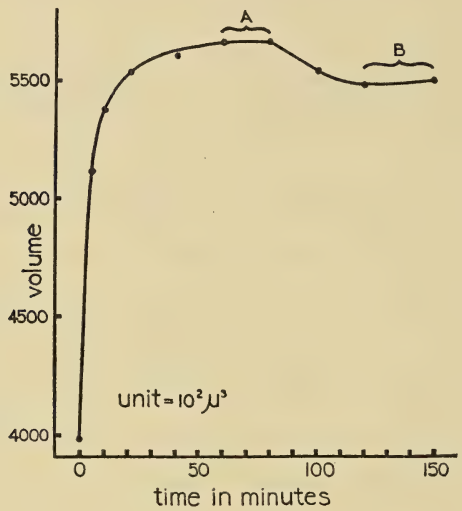
solvent volume of the eggs of *Echinometra lucunter*. Ignoring for the moment those values in the table marked with an asterisk (*), the significance of which will be considered below, there remain six values determined by ocular measurements and four by photographic measurements. These agree closely, giving average values of 36.9 per cent and 36.2 per cent, respectively.

TABLE 4—*Summary of the non-solvent volume determinations of the eggs of Echinometra lucunter as obtained by ocular and photographic methods.*

Exp.	Type of experiment	*b* as per cent of V_o	Time in diluted sea-water	Temp.	Remarks on behavior of eggs when returned to normal sea-water.
		p. ct.	mins.		
2-11	Equilibrium, ocular.	36.5	60-84	28-30°	Good development.
		48.9*	128-148	28-30°	No development.
		34.0	60-80	28-30°	Good development.
		36.8	60-72	28-30°	Good development.
4-7	Equilibrium, ocular.	38.7	60-70	27-30°	Good development.
2-30	Equilibrium, photographic.	35.5	67	31°	Good development.
		38.3	60	32°	Good development.
4-20	Ocular observation of single eggs during swelling.	38.8	60-70	31°	Good development.
		49.3*	120-130	31°	No development and vacuolization occurred just prior to these measurements.
4-27	As for 4-20.	36.5	60-70	27-29°	Good development.
		45.4*	120-135	27-29°	As for second value of Exp. 4-20.
2-17	Equilibrium, photographic.	35.3	75	28°	Good development.
2-19	Equilibrium, photographic.	35.7	80	31°	Good development.
		47.3*	120	31°	No development.

The four values marked with an asterisk in table 4 represent the non-solvent volumes of eggs determined from observations made 2 to 2½ hours after their immersion in the dilute sea-water solutions. The mean of these four values, 47.7 per cent, is greater than the non-solvent volume calculated from measurements of eggs after only 60 to 90 minutes' exposure to the experimental solutions. This difference signifies that the observed volume at this time was less than it was earlier, presumably because some shrinkage had occurred in the interval between the two sets of measurements. In experiments No. 4-20 and No. 4-27, single eggs were isolated and their respective initial diameters measured. They were then immersed in a dilute sea-water solution. Measurements of their diameters were made with a filar micrometer at intervals over a period of 2½ hours. The successive volumes of one of these eggs are plotted against time in Graph 1. In order that all values from 2½ hours of observation could be shown on the graph, the initial portion of the curve has been somewhat condensed.

At Region A of the curve (at from 60 to 90 minutes), the volume remained constant over a period of approximately 20 minutes and from this equilibrium value a non-solvent volume of 36 per cent was calculated. Eggs returned to sea-water and fertilized at any time up to the end of this phase



Graph 1

of the swelling process developed normally. Another equilibrium condition is indicated in the curve at Region B (at from 120 to 150 minutes) and the non-solvent volume calculated at this time is 45 per cent. However, when eggs were returned to normal sea-water and fertilized at this time, they did not develop. Shortly after this time, eggs left in the dilute sea-water solution underwent general cytolysis. Thus between these two equilibrium conditions there was a period of actual shrinkage. During this period active vacuolization was observed. Relatively small vacuoles appeared in the interior of the egg and then increased in size as they migrated slowly to the periphery. Although it was not possible actually to see these vacuoles empty their contents to the outside, this was inferred from the fact that no accumulation of vacuoles could be detected at the periphery, even though additional vacuoles were continually migrating in that direction.

The formation of vacuoles which migrated to the surface and burst was reported by Just (1930) for *Nereis* eggs returned to normal sea-water from hypotonic solutions. This phenomenon of vacuolization as a means of adjusting the osmotic pressure of protoplasm by the elimination of water had already been noted by Lloyd (1926) in both *Spirogyra* and *Vampyrella lateritia.* Lloyd and Scarth (1926 believed that in these cases the vacuoles had an external lipoid film which was extensible. Protoplasm

may be induced to undergo vacuolization by exceedingly diverse conditions which are discussed by Heilbrunn (1928). On page 247 of this reference, Heilbrunn concluded from observations on the eggs of *Arbacia punctulata* that this type of film is like the membrane formed on naked protoplasm under many conditions. His evidence for this conclusion lies in the fact that both reactions—membrane formation and vacuolization—are produced by the action of the same reagents, namely: hypertonic solutions, lipoid solvents, hypotonic solutions, etc. Vacuolization is, therefore according to Heilbrunn, a case of an internal surface precipitation reaction.

An internal surface precipitation reaction does not, however, account for the shrinkage of the eggs during the latter part of the swelling process. At this time several possible mechanisms for this shrinkage may be suggested, although no experimental data are available at present either to support or disprove any of them.

(1) Excretion out of the egg of a salt solution having the same osmotic pressure as the external solution: Although this mechanism would require no osmotic work, the factors initiating and limiting this mechanism are not evident.

(2) Excretion of water against an osmotic gradient: This would be the case if the internal and external osmotic pressures were equal at the first equilibrium and if the vacuoles contained only water. This would require some osmotic work and source for the necessary energy. Furthermore, this line of reasoning leads to the peculiar and inexplicable conclusion that at the second equilibrium stage, the osmotic pressure of the egg protoplasm would be greater than that of the external solution.

(3) Increase in the osmotic pressure of the egg protoplasm: During swelling there might be such an increase due to one or more of the following changes: (a) Increased dissociation of weak electrolytes on dilution, (b) breakdown of protein-salt complexes, and (c) the production of metabolites. As a result at the first equilibrium, the internal and external osmotic pressures would be equal because of the relatively rapid intake of water, but inside the cell there might still be an abnormally high concentration of some constituent to which the cell was permeable. There would then be a gradual exosmosis of this constituent after the first equilibrium had been established and a gradual decrease in the osmotic pressure of the egg protoplasm. Water would, therefore, have to leave the egg so as to maintain an osmotic balance at the second equilibrium.

Experiments on this phase of the swelling of cells are being started. The explanation of this vacuolization must, consequently, be left until further data are obtained on the eggs of this sea-urchin. This phase of the experimental results points to the necessity of continually observing the eggs during the swelling or shrinking processes and not just at the time of making measurements, so that all changes besides those of volume increase and decrease may be noted. Although Just (1930) thinks that the theories for

the water exchanges of cells should be modified in the light of the fairly general appearance of vacuolization, it should be noted in *Echinometra* that vacuoles are only formed by eggs which show by tests of their capacity for fertilization and development that they are no longer normal. Therefore for the present, at least, we must consider that the uninjured eggs of *Echinometra lucunter* are simple osmotic systems as proposed by Lucké and McCutcheon (1932) for the eggs of *Arbacia punctulata.*

SUMMARY

1. It has been shown that photography can be employed in the study of the water exchanges of living cells.

2. The non-solvent volume of the eggs of *Echinometra lucunter* is 36 per cent when calculated after from 60 to 90 minutes' exposure to experimental solutions.

3. Longer exposures to the experimental solutions result in a higher non-solvent volume of 48 per cent which is associated with pronounced vacuole formation.

4. The appearance of vacuoles after the attainment of the first equilibrium is discussed and three different explanations proposed.

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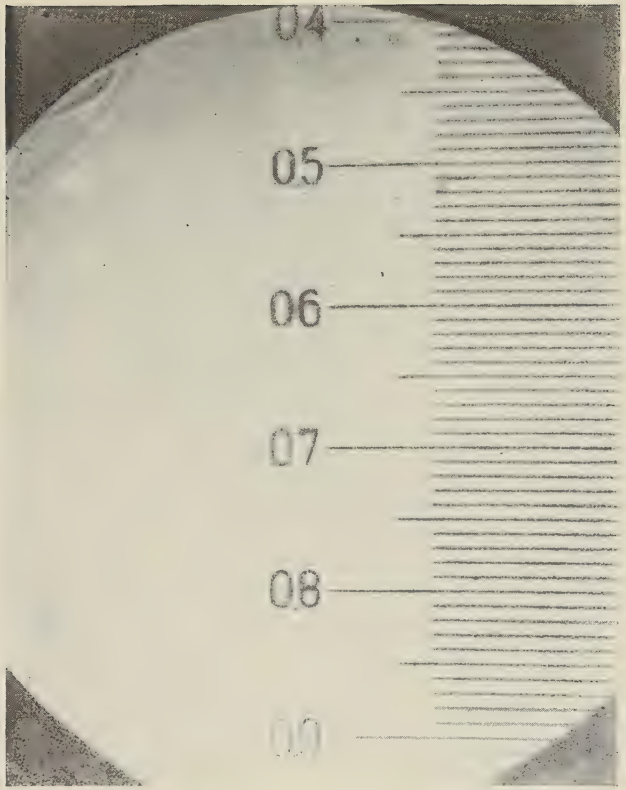

FIG. 1—Calibration photograph employing a 6xb ocular and a No. 6 objective.

FIG. 2—Calibration photograph employing a 6xb ocular and a No. 5 objective.

PAPERS FROM TORTUGAS LABORATORY, VOL. XXIX

XIV

ELECTRICAL RESISTANCE OF VALONIA

I: Changes in Resistance with Time in Impaled Cœnocytes

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One plate and four text-figures

[Issued March 10, 1936.]

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ELECTRICAL RESISTANCE OF VALONIA

I: Changes in Resistance with Time in Impaled Cœnocytes

INTRODUCTION

During the summers of 1933 and 1934 the writer was given opportunity to study at the laboratory of the Carnegie Institution of Washington located on Loggerhead Key, Dry Tortugas, Florida. Several species of *Valonia* were found growing abundantly on the various coral reefs of the Dry Tortugas archipelago. It seemed then that additional and useful information could be obtained from a study of the electrical resistance of impaled *Valonia* cœnocytes by using a technique more highly refined than that employed by previous investigators. *V. ventricosa* has been used by various investigators in their studies on bioelectrical phenomena, electrical resistance, and permeability, consequently many characteristics of this organism are quite well known.

The cœnocytes of *V. ventricosa* were impaled on the microtip of a salt-bridge with the aid of a micromanipulator. The insertion of a micro-capillary through the cœnocyst and protoplasmic layer into the vacuole obviously produces certain changes in the nature and properties of the protoplasmic layer. A study of these reversible injuries and their effect on electrical resistance was made. In this report an attempt is made to explain these changes in resistance with time on the basis of visible morphological changes which occur during the recovery of the protoplasmic layer.

Although only *V. ventricosa* was used in this study, the methods developed and used here may be extended to the study of other species of *Valonia*. The results obtained from a study of *V. ventricosa* will serve as useful lines of departure for the study of *V. macrophysa* and *V. ocellata* (species occurring at Tortugas) and for other species occurring elsewhere.

The writer is deeply indebted to Dr. S. C. Brooks and to Dr. M. M. Brooks of the University of California for their many helpful and critical suggestions.

A Leitz microscope, a modified Taylor micromanipulator, and various electrical instruments were generously provided by the Department of Zoology, University of California.

APPARATUS AND METHODS

THE CAPILLARY TECHNIQUE IN BIOELECTRICAL MEASUREMENTS

The capillary technique has been used by numerous investigators in their work on bioelectrical phenomena. Damon (11) and Osterhout, Damon, and Jacques (25) used this method in studying potentials of *V. macrophysa*.

Blinks (6), Taylor and Whitaker (35), and Kopac (20, 21) have used the capillary technique in studying the electrical conductivity and bioelectrical potentials of *V. ventricosa.* Umrath (38, 39, 40), Taylor and Whitaker (36), Gelfan (16), and Brooks and Gelfan (7) used the same technique in studying bioelectrical phenomena in *Nitella.*

These investigators have used two general methods in impaling their cœnocytes. Blinks, and Osterhout, Damon, and Jacques impaled their cœnocytes by hand. Damon placed his cœnocytes on suitable cork holders before impaling them. Blinks later used the same technique in studying *Halicystis.* Other investigators, namely, Taylor and Whitaker, Gelfan, Brooks and Gelfan, Umrath, and Kopac inserted the microcapillary into the cœnocyte by means of a micromanipulator.

Damon used "a fine glass capillary tube filled with artificial sap" and Osterhout, Damon, and Jacques specified that "the capillary should be very fine, with sides as nearly parallel as possible, and that the glass at the tip should be very thin." Blinks working with *V. ventricosa* stated that "the capillaries usually projected 2 or 3 mm. into the vacuole; they were about 1 cm. long and had an outside diameter of 0.2 to 0.5 mm." Blinks (2) working with *Halicystis* used "capillaries, drawn on the ends of quarter inch glass tubing, . . . usually about 0.5 mm. in outside diameter, and from 0.5 to 1 cm. in length." Taylor and Whitaker used microcapillaries of the dimensions used in microinjection technique. Brooks and Gelfan also used exceedingly small capillaries, approximately 0.005 mm. in external diameter.

Kopac (20) found that micropipettes as large as 0.1 mm. in diameter may be inserted into a *Valonia* cœnocyte without causing irreversible injuries. It was further found that if a normal cœnocyte was punctured with a glass or quartz capillary over 1 mm. in external diameter, a series of processes was initiated which resulted in the production of minute spheroidal bodies, the aplanospores.

The microcapillaries used in the following series of experiments were between 0.025 and 0.1 mm. in diameter. The insertion of these capillaries by means of a micromaniplator did not lead to any irreversible injury, namely, aplanospore production, in the *Valonia* cœnocyte.

MICROMANIPULATORS AND MICROMANIPULATION

The development of micromanipulators (see literature by Barber (1), Kite (19), Chambers (9), Peterfi (26), Taylor (34), Fitz (15), *et al.*, has enabled investigators to control various microinstruments with sufficient precision to remove cellular inclusions and to inject solutions into a living cell while these operations are observed microscopically. Details of the use of the micrurgical method are adequately discussed in the papers of Chambers (8, 10), Peterfi (26), Latter (22), Seifriz (28), and others.

Both the Chambers and Taylor micromanipulators were available for this work. The original Taylor micromanipulator (as described by Taylor

(34) and built by Arntzen) was not entirely satisfactory. In this instrument, precision control units are mounted on both the right and left sides of the microscope. Interference with the operation of the mechanical stage makes this position of units unsuitable. The precision control units, however, are satisfactory for this type of work. Their working range is ample, and their strength and stability permit the use of relatively large electrode vessels and other accessories (described below).

A new base for the Taylor micromanipulator was constructed so that the precision control units could be mounted in front of the microscope. A special holder which was mounted on each unit carried the electrode vessel (see Plate 1, D). In addition, a guide plate was mounted on the base of the micromanipulator so that the microscope could be replaced at the exact point from which it was removed.

ELECTRODES AND SALTBRIDGES

An important part of the work was the design and construction of suitable electrodes. In the literature these microinstruments are frequently referred to as microelectrodes. Blinks (6), however, pointed out that all non-polarizable so-called microelectrodes should be called microsaltbridges. The capillary point which is to be inserted into a cell is filled with an electrolytic solution and this leads to an electrode of more or less ordinary size. The capillary shank plus its shank makes up the microsaltbridge.

It is obvious that the metallic microelectrodes, as designed by Taylor (33) and Sen (29), can not be used for direct current measurements where solutions of electrolytes are involved. Various kinds of microsaltbridges have been described (see papers by Taylor (33), Ettisch and Peterfi (13, 14), Gelfan (16), and Telkes (37).

Telkes using the Hg, Hg_2Cl_2 electrode designed a microsaltbridge in which the capillary was filled with an electrolytic fluid instead of a conductive gel. She was able to control the flow of fluid in the capillary by means of a Luer syringe. This arrangement seemed to be very logical and was to be preferred to the other types of saltbridges which were filled with a gel.

All previously described electrode systems were tried in this work. An apparatus was finally designed by the writer which combined a Chambers (9) microinjection unit with a calomel half-cell. Particular attention was paid to the design of the microinjection apparatus so that one could change and control the fluid within the saltbridges at will. It was also necessary to design a system which could be mounted on a micromanipulator. The final design is described below.

The electrode vessel (see Plate 1, A)—The main shank of the electrode vessel (1) was made from a piece of 8-mm. pyrex capillary tubing having a 2-mm. bore. This shank when completed was about 12 cm. long. A piece of pyrex tubing 1 cm. in diameter and about 4 cm. in length was

sealed to one side of the capillary tube forming the stem (2). Another pyrex tube (3) about 6 cm. long which would form a sliding fit over the stem (2) was sealed at one end, and in this end was sealed a piece of platinum wire (4). An adaptor (5) to accommodate a Luer needle was cemented to the shank (1) with hard de Khotinsky cement. The opposite end was connected to the microinjection apparatus.

The stem (2) was filled with a 3 per cent solution of agar saturated with KCl. The main bore of the shank was kept open at this point so that fluid could be forced from one end to the other. This agar plug prevented a too rapid diffusion of KCl from the main body of the electrode to the saltbridge fluid. A layer of very clean mercury was put into the tube (3) so that the projecting platinum wire was completely covered. A column of specially prepared electrolytic calomel (La Motte), about 1 cm. high, was placed on the mercury. The tube was then completely filled with a saturated KCl solution. Finally the vessel was assembled by inserting the stem (2) into the tube (3), being careful not to introduce air bubbles between the agar and the saturated KCl solution. The stem and tube were sealed together with a mixture consisting of equal parts of purified beeswax and resin. The complete electrode system consisted of the following components: saltbridge fluid/ saturated KCl-agar/ saturated KCl/ saturated Hg_2Cl_2/ Hg/ platinum.

A 2 cc. Luer syringe was used to control the fluid within the saltbridges. In most cases the connection from the electrode vessel to the syringe was a piece of pyrex tubing; but where the syringe was not mounted directly on the micromanipulator unit, a connection was formed by a piece of rubber pressure tubing (see Plate 1, D).

Either natural or artificial sap of *V. ventricosa* was used in the microsaltbridge. The entire system had to be filled with this fluid. The syringe was filled to its full capacity so that the fluid could then be forced from the syringe into the shank (1) of the electrode vessel. A microsaltbridge was fastened to the adaptor at (5). With the saltbridge in place, fluid was forced into the capillary, thereby completely filling the shank and the saltbridge. If any air bubbles were seen, the process was repeated.

The saltbridges—Two types of glass saltbridges were used in this work. A large or macrosaltbridge (see Plate 1, B) was used to establish electrical continuity between the calomel half-cell and the external medium surrounding the organism. This saltbridge was usually 2 mm. or more in diameter and it was bent at right angles near the tip as shown in the photograph. The microsaltbridge was used to establish electrical continuity between the interior of the organism and the other calomel half-cell.

Because of the mechanical difficulties involved in piercing the cellulose cœnocyst, the microsaltbridges had to be properly designed and constructed. After numerous experiments it was found that by drawing the end of a glass tube approximately 2 mm. in diameter abruptly to 0.2 mm. or so in

diameter, a microtip could be easily drawn out in the flame of a microburner. The tip was then sufficiently strong and sharp to penetrate the cœnocyst. The sides of the microtip need not be parallel as specified by Osterhout, Damon, and Jacques (25). The sides of the microsaltbridge described in this report were tapered, thereby forming a wedge-shaped tip. There was no danger of a cœnocyte falling off of this microtip, because the cœnocyte was supported by its containing vessel (described below).

After the tip of the microcapillary was made, the saltbridge was bent about 45° near the tip (see Plate 1, C). Then the saltbridge was cut to the correct length and sealed to the base of a Luer needle with hard de Khotinsky cement. This saltbridge was now ready to be shipped on to the adaptor of the electrode vessel.

The completed electrodes were frequently tested by the following method. Both saltbridges were filled with the same fluid and their tips dipped in a vessel containing some of the same solution. A current was passed through the system, and the potential was increased until a microammeter indicated a current flow of 100 microamperes. This current was kept flowing for several minutes and any change in current intensity was noted. In most cases, 100 microamperes of current could be kept flowing continuously without showing any appreciable change in resistance. The current flowing through the half-cells, while the protoplasmic resistance was being measured, rarely exceeded 25 microamperes.

It was important in making this test to have the solutions in the saltbridges and in the vessel identical in composition, otherwise changes in resistance might occur, due to the replacement of ions in the saltbridges with other ions possessing different mobilities and other characteristics. This matter is considered in greater detail below.

METHODS OF HANDLING VALONIA

Cœnocytes of *V. ventricosa* were collected from the leeward side of the Bird Key and Bush Key reefs, from the seaward side of Long-Bush Key reef, and from the pier at Loggerhead Key. No difficulty was experienced in obtaining an abundant supply during the months of June and July 1933 and 1934. The cœnocytes were brought in the laboratory and placed in fresh sea-water; 10 to 20 hours later the cœnocytes were cleaned by removing all bryozoa, foraminifera, sponges, and algæ that might be found growing on the cœnocyst. The cleaned cœnocytes were then stored in large volumes of fresh sea-water and the containers were kept covered so that direct light might be avoided. The sea-water was changed every 24 to 36 hours.

Although all sizes of cœnocytes were collected, only those 3 to 5 mm. in diameter were used. These small cœnocytes have a thin cellulose wall which may be quite easily pierced by the microsaltbridge. The larger

cœnocytes possess a cellulose wall which ordinarily is too tough to be punctured by a microcapillary.

Each cœnocyte was carefully examined microscopically and only those which showed a uniform distribution of chloroplastids were retained for further experimental work. If during the cleaning process some of the small cœnocytes were injured and examined microscopically, the chloroplastids appeared to be irregularly dispersed and frequently tiny vacuoles could be seen in the inter-chloroplastidal protoplasm. If these cœnocytes were stored in fresh sea-water away from direct sunlight, recovery usually took place. A later microscopical examination showed a uniform distribution of chloroplastids and a marked decrease in the apparent amount of inter-chloroplastidal protoplasm. On the other hand, the larger cœnocytes, if injured, rarely recovered.

METHOD OF IMPALING VALONIA

A small round glass petri dish 6 cm. in diameter and 1.2 cm. deep was cemented to a 1½ by 3 inch microscope slide. A piece of cork cut out to resemble a large "V" was cemented to the right side of this vessel with the open end of the "V" facing toward the center. The cœnocyte was placed in the open part of the V-shaped cork support and held in place there during the insertion of the microsaltbridge.

The slide holding the glass vessel and the cork support within it was placed on the stage of a microscope and this unit could be oriented in a horizontal plane by means of a mechanical stage. About 10 cc. of fresh filtered sea-water were placed in the vessel and a selected cœnocyte was then mounted inside the V-shaped support. The cœnocytes were handled with rubber tipped forceps.

After it was placed in the vessel, the cœnocyte was centered in the microscopical field by manipulating the mechanical stage. The microsaltbridge, previously mounted on the left unit of the micromanipulator and filled with vacuolar sap, was then oriented by using the micromanipulator until the tip was near the top and center region of the cœnocyte. The microsaltbridge was lowered until the tip was actually touching the cœnocyst. Then by manipulating the mechanical stage, the slide holding the vessel and cœnocyte was quickly moved toward the tip, and in this way the microsaltbridge was inserted into the vacuole with the minimum amount of injury.

After the cœnocyst was pierced, the microtip was pushed into the vacuole about 0.5 mm. or more by using the micromanipulator. The tip of the microsaltbridge was wedge-shaped, and it was inserted far enough into the vacuole so that it would fit the opening produced in the cœnocyst. The cœnocyte and microsaltbridge were held in place mechanically so that no further disturbance of the protoplasmic layer took place. This additional disturbance is difficult to prevent if the cœnocytes are impaled by hand.

Instead of using a hydrostatic pressure head to keep the cœnocytes inflated (see Blinks, Osterhout, *et al.*), a small pressure was created by gently compressing the Luer syringe. A small drop of sap was thereby forced from the microsaltbridge into the vacuole. This insured an electrical continuity between the fluid in the vacuole and the fluid in the microsaltbridge.

The possibility of contaminating the vacuolar sap by the injection of artificial sap has been discussed by Osterhout, Damon, and Jacques (25). They reported that the introduction of small amounts of artificial sap into the vacuole of *V. macrophysa* produced no significant changes in their experiments. In the experiments described below, no appreciable effects were noted after small amounts of artificial sap were injected into the vacuole of *V. ventricosa.*

METHODS OF MEASURING AND CALCULATING RESISTANCE

At Dry Tortugas, Florida, where all work on *Valonia* was done, a Wheatstone bridge circuit was used. The vacuum tube detector described by Blinks (3) was not available, but, as will be shown below, a detector having this sensitivity was not needed.

Several accessories have been added to the bridge. The power input circuit consisted of a 3-cell storage battery. This was shunted by a voltage divider so that the potential applied to the bridge could be regulated. A high resistance voltmeter was used to measure the potential (E_o) actually applied to the bridge.

The bridge proper consisted of a network of four resistance arms. Two General Radio type 133, 1000-ohm resistors were used for the ratio arms. A General Radio type 102L decade resistance box (R) was used as a standard. The fourth resistance (S) was a General Radio type 133, 10,000-ohm resistor. The unknown resistances (x), in this case the electrodes and the cœnocyte, were connected in parallel with the 10,000-ohm resistor. A Leeds and Northrup DPDT switch was used as a commutator for changing the direction of the current flowing through the unknown resistance. A Leeds and Northrup lamp and scale galvanometer having a sensitivity of 2.5×10^{-8} ampere per scale division was used as a detector. The circuits leading to the galvanometer and to the unknown resistance were opened and closed by a double contact key. The midpoint of the bridge was grounded.

Since the resistances (x) to be measured were between 80,000 and 150,000 ohms, it was found useful to shunt this high resistance with the 10,000-ohm resistor (S). Shedlovsky (30), while measuring very high resistances of conductivity cells, also found it expedient to shunt the conductivity cell with a 10,000-ohm resistor. The bridge then needs to handle resistance only up to 10,000 ohms. A galvanometer sensitive to 2.5×10^{-8} ampere per scale division used as a detector was sufficiently sensitive to record an off balance of 0.5 ohm when the EMF applied to the bridge was 5 volts.

If the values of the ratio arms (r), the galvanometer sensitivity (di), the voltage (E_o) applied to the bridge, and the magnitude of the resistance (R) to be measured are known, the sensitivity or minimum change in resistance (dR) that may be detected depends on the following relation: $dR = \frac{R(R + r)\ di}{E_o}$.

The resistance of the shunt was first measured. Then the resistance (R) of the parallel resistances (S) and (x) was measured. Since $R = Sx/S + x$, the value of the unknown resistance (x) may be calculated from $x = \frac{SR}{S - R}$. Or $\log x = \log SR - \log (S - R)$

Since the maximum sensitivity, dR, is 0.5 ohm for an applied potential of 5 volts and using a galvanometer sensitive to 2.5×10^{-8} ampere per scale division, the significant change in (x) may be calculated from $\log x = \log SR - \log (S - R)$. The following table gives the values of x from various values of R.

R	Log x	x
8749.5	4.94490	88,085
8750.0	4.94510	88,125
8750.5	4.94530	88,165

This table shows that if x has a value of approximately 88,000 ohms, an increase or a decrease of 40 ohms may be detected and any change greater than this is significant. This is a sensitivity of less than 0.05 per cent of the total resistance to be measured. Such sensitivities are more than adequate for biological investigations.

The large saltbridge or macrosaltbridge previously filled with fresh sea-water was dipped in the sea-water contained in the vessel holding the cœnocyte. As soon as the microsaltbridge was inserted into the vacuole, the resistance was measured. The first measurement is designated as resistance at time = 0 and this corresponds to the start of the experiment. The discussion of the resistance values and their calculation follows below.

The resistance of the chain: *microsaltbridge/ sap/ sea-water/ macrosaltbridge* and the half-cells associated with the saltbridges is designated as the basal resistance, Rb. This resistance is determined before the microsaltbridge is inserted into the vacuole.

While the basal resistance is being measured, the microsaltbridge must be kept positive. The microsaltbridge is filled with natural or artificial sap (0.57 m. KCl plus 0.03 m. NaCl) of *V. ventricosa*. This sap contains approximately 95 per cent of K^+ while the sea-water contains over 85 per cent of Na^+. The absolute velocity of the K-ion is 6.7×10^{-4} cm.^{1} sec.$^{-1}$ volt-cm.$^{-1}$, while the absolute velocity of the Na-ion is 4.5×10^{-4} cm.^{1} sec.$^{-1}$ volt-cm.$^{-1}$. If the microsaltbridge is negative, the less mobile Na-ion would replace the more mobile K-ion within it, thereby increasing the resistance of the electrode system. This point has been clearly emphasized by Blinks (5). On the other hand, the concentration of K-ions leaving

the capillary and entering the sea-water (assuming that the capillary is positive) would be too small to produce an appreciable change in the resistivity of the sea-water. This has been checked experimentally. Since the anions of sea-water are mostly Cl-ions, no appreciable change in resistance is expected with a positive microsaltbridge, because the Cl-ions in this saltbridge will be replaced almost entirely by other Cl-ions coming in from the sea-water.

However, if sap is substituted for the sea-water in the vessel and in the saltbridges, no difference in resistance will result, even though the microsaltbridge may be negative. Using sap in place of sea-water reduces the chain to the following: *microsaltbridge/ sap/ macrosaltbridge.* The concentration of K-ions in the microsaltbridge end in the sap is approximately the same. Even if the capillary were made negative, the cations already present would be replaced by the same concentrations of K- and Na-ions. There would be no opportunity for the K-ion with its high mobility to be replaced by Ca, Mg, or other cations having different mobilities and other characteristics.

The total resistance, Rt, includes the resistance of the chain: *microsaltbridge/ sap/ protoplasm/ cœnocyst/ sea-water/ macrosaltbridge.*

The increase in the total resistance, Rt, above the basal resistance, Rb, is attributed to the resistance to the flow of direct current offered by one layer of protoplasm and the cœnocyst. This resistance is designated as Rp. Thus: $Rp = Rt - Rb$.

The cœnocyst is laminated in structure and is predominately composed of cellulose (see also Doyle, 12). Osterhout (24) has shown that this wall is freely permeable to many salts. He stated that an applied salt solution quickly penetrated the wall and came in contact with the external surface of the protoplasm. Damon (11) pointed out the difficulties in measuring potential differences across the protoplasm in such marine algæ as *Valonia*, "where the cell wall is imbibed with a highly conducting solution (sea-water) and hence has a low electrical resistance."

Blinks (3) reported that the resistance of an intact *Valonia* cœnocyte in which the protoplasmic layer was destroyed was from 1.0 to 2.6 per cent of the resistance of the living intact cœnocyte. This resistance corresponds very closely to that of the extracted vacuolar sap. He further reported that the resistance of the cell wall (cœnocyst) did not vary with applied potential or direction of current flow. In a later publication (4) he reported that there was no increase of total resistance over basal resistance in cells (cœnocytes) killed by chloroform.

The resistance of a cœnocyte in which the protoplasmic layer has broken down to form aplanospores shows no appreciable increase over the original basal resistance.[1] It seems fairly certain that if the cœnocyst is imbibed with sea-water, vacuolar sap, or some other strong electrolytic solution,

[1] Second paper of this series, now in preparation.

it would offer no appreciable resistance to the flow of direct current. There is no increase in resistance of the cœnocyst with time. The dry, well-washed air-filled cœnocyst is entirely different, and according to Blinks (3) this does offer considerable resistance to the flow of an electric current. Damon (11) reported that the conductivity of the cœnocyst could be decreased by leaching with distilled water.

The Rp value, except for a very small fraction, which may be neglected, is the resistance to the flow of direct current offered by one layer of protoplasm.

EXPERIMENTAL DATA AND DISCUSSION

PREVIOUS WORK BY OTHER INVESTIGATORS

Taylor and Whitaker (35) were able to measure potential differences of only 0.002 volt between the vacuolar sap and sea-water shortly after a microsaltbridge was inserted into the vacuole of *V. ventricosa.* Osterhout (23) also measured potentials of 1 or 2 millivolts in newly impaled *Valonia* cœnocytes. Jost (18, 19) repeated these experiments and got similar results. Umrath (38) using *Nitella* was able to measure only exceedingly small potentials from the newly impaled cœnocytes. Later Osterhout, Damon, and Jacques (25) found that if the cœnocytes were given time to recover, much higher potentials could be obtained.

Blinks (6) found that the effective resistance of newly impaled *Valonia* cœnocytes was so low as to be negligible at all current densities. He attributed this low resistance to electrical leaks around the inserted capillary and also to more profound changes in the entire protoplasmic surface. Osterhout, Damon, and Jacques (25) reported that when impaled cœnocytes gave a high potential, the cœnocyte would not only cling to the capillary (a mechanical seal), but that a ring of dark material would be deposited around the capillary.

Blinks (6) did not present data which described all changes in resistance with time in impaled *Valonia* cœnocytes. He has, however, published values of the back EMF in the protoplasmic layer. According to Blinks this value is equal to E_0 (Rp/Rt), where Rp = "apparent" resistance of protoplasm, Rt = protoplasmic resistance plus resistance of capillaries, and E_0 = potential applied to the cœnocyte. Blinks (6) has determined the back EMF, by direct reading with a calibrated electrometer, or by measurement of the galvanometer deflection at the instant of break, as recorded photographically. The value, E_1 (the back EMF) may also be calculated from: $E_1 = \frac{(Rt - Rb)\ Eo}{Rt}$. Here: Rt = Rb + Rp.

Because of the close similarity between the calculated values of E_1 and the back EMF's as measured by various means, Blinks believes that the apparent rise in resistance is due to a polarization potential. It is, of course, physically impossible to measure these potentials during the time

a current is flowing through the protoplasm, but Blinks measured the potentials immediately after a current was passed through the cœnocyte. Consequently, Blinks may be correct in maintaining that the decrease in current which is measured as an increase in resistance is due to a polarization potential.[1] On the other hand, it is quite possible that there is a decrease in the mobility of the ions passing through the protoplasm, thereby producing an actual increase in resistance.

In the present state of our knowledge regarding the flow of current through protoplasm, we are only able to state that the resistance or the apparent resistance may be the possible result of at least two factors, namely, the production of a counter EMF and/or a decrease in the mobility of the ions passing through the protoplasm. It is not possible at present to evaluate the exact effect of each factor on the resistance of protoplasm. Blinks data will be considered here because these back EMF values are related to the protoplasmic resistance and they indicate the direction and time of change.

Immediately after impalement, Blinks found that the back EMF ranged from 0 to 17 millivolts. Shortly after impalement this range was from 0 to 95 millivolts. The back EMF in partially recovered cœnocytes was from 11 to 155 millivolts, while in the constant state the back EMF dropped to a lower range of 16 to 80 millivolts. These data are not very specific as to time, but they do show that, as the cœnocytes recover, there is an apparent increase in resistance.

Blinks' data show that recovery from impalement in *Valonia* cœnocytes is a slow process. However, Blinks used microcapillaries ranging from 0.2 to 0.5 mm. in external diameter which were inserted into the vacuole by hand. It seemed to the writer that additional significant data could be obtained from a study of impaled *Valonia* cœnocytes if smaller capillaries, mechanically inserted and held in place, were used. Furthermore, information concerning the changes in resistance with time in impaled cœnocytes would be more useful if the time were expressed in definite intervals.

EXPERIMENTAL DATA AND DISCUSSION OF PUNCTURES

The changes in resistance with time in 28 cœnocytes were selected from the measurements made on over 100 cœnocytes. All these cœnocytes were about 0.5 cm. in diameter, spheroidal, and dark green in color. Many more cœnocytes were used in this investigation, but frequently the fragile microsaltbridge was broken while its tip was inserted into the vacuole. Occasionally the lumen of the microsaltbridge became clogged with protoplasm or other material.

The microtip of the saltbridge was inserted into the vacuole by means of a micromanipulator. It was difficult to tell whether any sap escaped dur-

[1] Blinks' theory will be further considered in another paper, now in preparation (third paper of this series).

ing this process, and in all probability a trace of sea-water was introduced into the vacuole with the insertion of the microtip. The macrosaltbridge was immersed in the sea-water contained in the vessel holding the cœnocyte.

A careful study was made during the summer of 1934 regarding the types of visible changes produced in a cœnocyte when punctured with a micro-

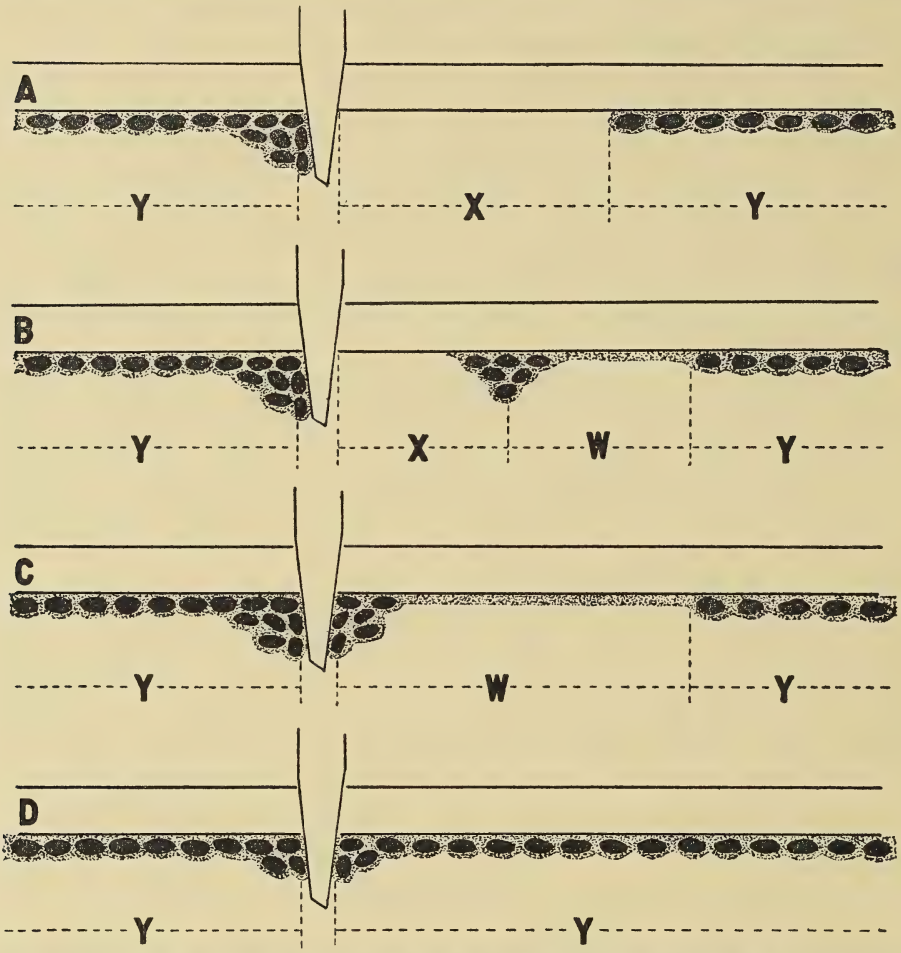

Fig. 1—Diagram showing relation of a microsaltbridge to cœnocyst and protoplasmic layer. This is a cross-sectional view of the protoplasmic layer, the structure of which is highly idealized. The various zones in a type B puncture are represented. Chloroplastids are solid black, the hyaline protoplasm is stippled and the cœnocyst is white.

saltbridge (see Kopac, 21). The resulting puncture depends (1) on the sharpness and size of the microtip, and (2) on the setting of the microtip relative to the cœnocyte prior to its insertion.

If a sharp and small microtip was set perpendicular to the periphery of a cœnocyte, a clean puncture (type A) would result when the cœnocyte was pushed against the microtip. No tearing reaction in the protoplasmic layer near the microtip was induced. The protoplasmic layer surrounding the

microtip appeared to be nearly normal as indicated by the very slight disturbance of chloroplastids.

If the microtip was not set perpendicular to the cœnocyte there would be a lateral as well as a vertical displacement during its insertion, thus producing a different type of puncture (type B). In this case there was a slight tearing reaction in the protoplasmic layer near the tip of the capillary, producing a circular or elliptical opening through this layer, frequently 0.02 to 0.03 cm. in diameter. Careful microscopical study revealed only a few strands of hyaline protoplasm in this region. As the protoplasm migrated toward the microtip, this torn area was soon eliminated (in 5 to 10 minutes), but the new protoplasmic zone was hyaline in appearance, due to the lack of chloroplastids (see fig. 1, C). Complete recovery involved a redistribution of chloroplastids which resulted in an appearance not unlike that of the normal, uninjured cœnocyte (see fig. 1, D).

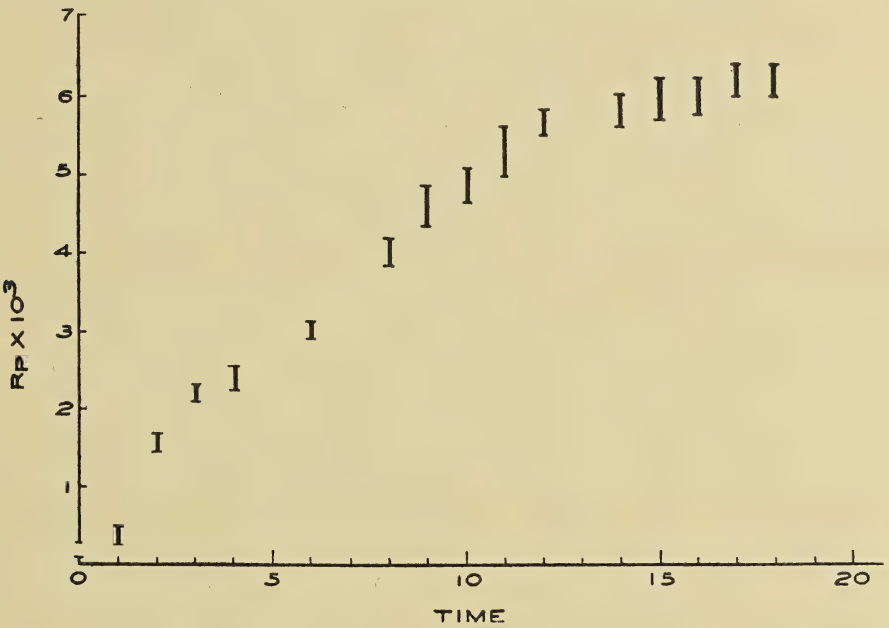

Fig. 2—Graph showing changes in resistance with time in type B punctures. Values were taken from table 1. The Rp and time are given in ohms and minutes, respectively.

More drastic punctures (type C) were produced when microtips over 0.02 cm. in diameter were used. Type C punctures were produced when the cœnocytes were punctured by hand. The resulting injuries were very severe and a considerable tearing reaction was induced in the protoplasmic layer. These injuries were frequently irreversible, and the protoplasmic layer was apt to break down to form aplanospores (see Kopac, 20). The

results of this series will be discussed in another paper, now in preparation.[1]

As soon as the microsaltbridge was inserted into the vacuole, the resistance of the system was measured. Then at intervals of one or two minutes the resistance was again measured until a constant resistance level was reached. The positive current was lead from the microsaltbridge into the sap, through the protoplasmic layer into the sea-water, and finally through the macrosaltbridge. The microsaltbridge was kept positive, thereby causing the migration of cations toward the macrosaltbridge.

The basal resistance, Rb, was determined both before and after the resistance of the cœnocyte was measured. Whenever there were appreciable discrepancies between the two readings, the data were discarded. Obviously, other factors besides protoplasm were responsible for these changes in resistance.

TABLE 1—*Type B punctures*

Time	Number of readings	Average Rp in ohms	Average Rp in per cent
0	28	120 ± 40	2
1	8	390 ± 120	6
2	15	1620 ± 100	26
3	7	2260 ± 110	36
4	20	2440 ± 120	39
5	0	....	..
6	16	3060 ± 110	49
7	0	....	..
8	16	4060 ± 100	65
9	10	4650 ± 250	74
10	14	4970 ± 200	79
11	6	5420 ± 300	86
12	16	5750 ± 150	92
13	0	....	..
14	11	5910 ± 200	94
15	15	6050 ± 250	96
16	24	6130 ± 150	97
17	21	6270 ± 200	100
18—25	21	6270 ± 200	100

The study of type B punctures gave very interesting results. These results are summarized in table I and in the graph shown in figure 2.

There are several significant points shown in this study. At $T = 0$, the start of the experiment, Rp was only slightly higher than the resistance of the sea-water, namely, Rb. The average Rp at $T = 0$ was 120 ± 40 ohms. This is about 2 per cent of the ultimate constant resistance level. During the first minute there was a slight increase; the Rp at $T = 1$ being 390 ± 120 ohms. The greatest increase in resistance (from 390 to 2260 ohms) occurred between the first and third minute. The resistance then

[1] Second paper of this series.

continued to increase until a steady or constant value of 6270 ± 200 ohms was attained. This constant level was reached in an average time of 16 to 18 minutes after the microsaltbridge was inserted into the vacuole. At 6 minutes the average resistance was 3060 ± 110 ohms; this quantity being approximately half of the ultimate constant Rp value. It is interesting to note that calling the ultimate resistance 100 per cent, it takes nearly twice as long for the resistance to change from 50 to 100 per cent as it does to reach the initial 50 per cent increase.

This study indicates that at least two factors operate to produce these changes in resistance with time. As might be expected, the closure of the gap in the protoplasmic layer is the predominant factor governing the changes occurring during the first six or seven minutes. If the torn area in the protoplasmic layer is healed and under microscopical examination is shown to remain so, it is obvious that a further increase in Rp must be due to some other factor. No explanation for the series of changes occurring between the sixth and eighteenth minute was apparent at first.

EXPLANATION OF RESISTANCE CHANGES

We may assume that there are, at first, two general paths for the current to flow in a type B impaled cœnocyte. A fraction of current, Ix, may pass through the more or less circular zone x in which there is only a trace of hyaline protoplasm. Most of this current would pass unimpeded through the cœnocyst (see fig. 1, A). The other fraction of current, Iy, would pass through the normal, uninjured portion of the protoplasmic layer, zone y.

The magnitudes of Ix and Iy would be E/Rx and E/Ry, respectively, where E is the potential applied to the cœnocyte, and Rx and Ry are the resistances of zone x and zone y, respectively. The total current, Ip, flowing through the cœnocyte would be $Ip = \frac{E}{Rp} = \frac{E}{Rx} + \frac{E}{Ry}$. The total resistance of the cœnocyte, Rp, is actually measured. If we let $E = 1$, then Rp will be given by the expression

$$\frac{1}{Rp} = \frac{1}{Rx} + \frac{1}{Ry} \qquad (1)$$

Unless the entire cœnocyte is injured we may assume that the characteristics of the normal protoplasm do not change appreciably, and Ry may be assumed to remain constant. Some factors which may affect the quantity Ry will be treated below. The limiting value of Rp will be Ry and this is attained when $1/Rx = 0$. Shortly after the cœnocyte is impaled the protoplasm begins to migrate toward the microtip. There may be a lag of 0.5 to 2 minutes before this migration begins. (Note: the relatively slight increase in Rp from $T = 0$ to $T = 1$.) As the cœnocyst heals, zone x becomes replaced by protoplasm so that the current is no longer able to pass directly through the cœnocyst (see fig. 1, B). The resistance,

Rx, increases rapidly, due to the rapid decrease in the area of zone x and the measured Rp is thereby increased. The resistance, Rx, may be expressed as follows:

$$Rx = \frac{rX \quad L}{Ax} \tag{2}$$

In this expression rX represents the resistivity of the cœnocyst which is nearly the same as that of sea-water. The thickness of the cœnocyst, L, lies usually between 0.001 and 0.003 cm. Ax represents the area of the torn portion of the protoplasmic layer, namely, zone x.

If the value for Rx in equation (2) is substituted in equation (1), then

$$\frac{1}{Rp} = \frac{1}{Ry} + \frac{Ax}{rX\ L} \tag{3}$$

As the cœnocyte heals, the area, Ax, will decrease and from equation (3) it is obvious that there will be an increase in Rp. The rate with which Rx increases varies from cœnocyte to cœnocyte. On the average one may expect the elimination of zone x within 5 to 10 minutes after the puncture is produced. Usually the larger the opening the longer it takes for this zone to become extinct. When the torn area is repaired, the area, Ax, becomes zero, and theoretically the resistance, Rx, becomes infinite.

But even with the complete closure of this torn area, the resistance, Rp, continued to increase. In order to explain the later increase in resistance it will be necessary to consider the cytology of *Valonia.*

The structure of the protoplasmic layer in *Valonia* is unique. The protoplasm is arranged in the form of a delicate sheet lining the inside of a cellulose wall. According to Doyle (12), there is a continuous hyaline cytoplasmic surface beneath the cellulose wall and another at the surface of the central vacuole. Chloroplastids, lipoid granules, and nuclei are imbedded in the hyaline cytoplasm bounded by its two surfaces.

The chloroplastids, many of which contain a starch grain (see Doyle, 12), are flattened ellipsoidal bodies; the average diameter being 6 to 8μ and the thickness 3 to 5μ. In the healthy cœnocyte the chloroplastids are uniformly distributed and only one layer is present. The thickness of the protoplasmic layer varies between 5 and 8μ. The nuclei are extremely numerous, yet Doyle found that these bodies only constitute about 1.2 per cent of the total cytoplasmic volume. The hyaline protoplasm fills in the spaces between the chloroplastids and other bodies so that in *Valonia* we have essentially a layer of chloroplastids imbedded in a delicate sheet of hyaline protoplasm.

In many cœnocytes considerable amounts of inter-chloroplastidal hyaline protoplasm may be seen. This condition may be due either to the reduced number of chloroplastids or to their orientation within the protoplasmic layer. In dark-green cœnocytes there is very little inter-chloroplastidal hyaline protoplasm. The distances between chloroplastids in these dark-green cœnocytes are frequently less than 1μ. In pale-green cœnocytes,

there is considerably more inter-chloroplastidal hyaline protoplasm; the distances between chloroplastids may be from 3 to 5μ

The constant resistance, Ry (see equation, 13), of cœnocytes having a great many chloroplastids and a negligible amount of inter-chloroplastidal hyaline protoplasm is approximately 2.5 times higher than the Ry of cœnocytes having an appreciable amount of inter-chloroplastidal hyaline protoplasm (see table 2).

For purposes of approximation, we may consider the protoplasmic layer in *Valonia* to be a flat sheet of hyaline protoplasm extending over an area, A, and of thickness, L. In the protoplasm we have a quantity of chloroplastids where their thickness approximates L. Thus in A of figure 3, we may represent the condition of the protoplasm in a dark-green uninjured cœnocyte. This condition shows a very high density of chloroplastids and

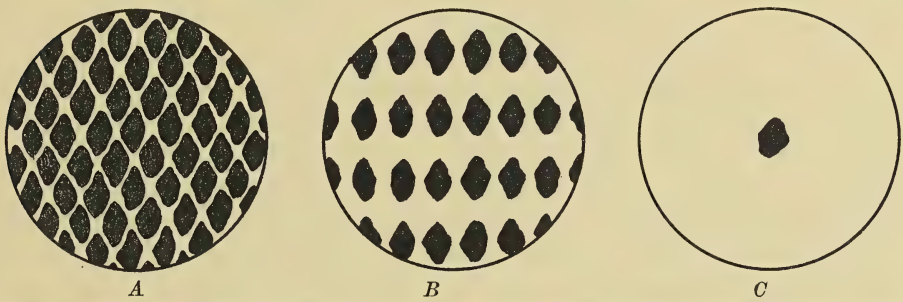

FIG. 3—Diagram showing surface views of cœnocytes with varying chloroplastid content and the relation between chloroplastid density and inter-chloroplastidal hyaline protoplasm. Chloroplastids are solid black; inter-chloroplastidal hyaline protoplasm is white.

the fraction of the total area consisting only of hyaline protoplasm is very small. In B of figure 3 is represented the condition of the chloroplastids in a pale-green cœnocyte. The density of chloroplastids is lower, and the inter-chloroplastidal hyaline protoplasm represents a greater fraction of the total area. In C of figure 3 we have a condition present in the protoplasm which has replaced the gap produced by the insertion of a microsalt-bridge. The chloroplastids are few in number and most of this area consists of hyaline protoplasm.

The approximate areas occupied by the chloroplastids in the protoplasmic layer have been measured. Small areas of a cœnocyte were drawn by Doyle (12) on graph paper with the aid of a camera lucida and the relative areas occupied by the chloroplastids determined. He found that on the average the chloroplastids occupied 67 per cent of the total area of the protoplasmic layer.

Individual cœnocytes of *V. ventricosa* were similarly measured by the writer. More than 0.9 of the total area was occupied by chloroplastids in a dark-green cœnocyte while in a pale-green cœnocyte the chloroplastid fraction represented approximately 0.5 of the total area. In the newly repaired

zone (w in fig. 1, C), over 0.99 of the total area consisted of hyaline protoplasm.

There is evidence that the chloroplastids consist largely of lipoidal and other non-conducting substances (see Spoehr, 31, pp. 378-382). One would expect the flow of ions through these more or less lipoidal bodies to be greatly restricted, if not inhibited entirely. If we postulate that the current which passes through the protoplasmic layer is able to pass only through the hyaline protoplasm, we may then begin to interpret some of the changes in resistance which occur in *Valonia*.

Let us consider a hypothetical *Valonia* cœnocyte, namely, a cœnocyte consisting entirely of hyaline protoplasm without chloroplastids. We may further assume that the current will flow uniformly through this layer of hyaline protoplasm. The resistance of a sheet of hyaline protoplasm of area, A, and of thickness, L, would be

$$R = \frac{rP \quad L}{A} \tag{4}$$

Here rP represents the resistivity of the hyaline protoplasm.

If we now consider an actual *Valonia* cœnocyte, namely, one with chloroplastids, and if we still postulate that only the hyaline protoplasm is capable of conducting a current, we may demonstrate mathematically that an increase in resistance may follow from an increase in chloroplastid density.

Let q represent the fraction of the total area, A, of the protoplasmic layer occupied by chloroplastids. The remaining fraction $(1 - q)$ represents the fraction of A capable of conducting a current, that is, the fraction of hyaline protoplasm. Including the value $(1 - q)$ in equation (4), we now have an expression which includes the factor of chloroplastid density and its effect on resistance.

$$R = \frac{rP \quad L}{A(1 - q)} \tag{5}$$

In the hypothetical *Valonia*, in which $q = 0$, the resistance, R, would be $R = \frac{rP \quad L}{A}$. The resistivity of the protoplasm is again represented by rP.

In condition C (fig. 3), $q = 0.01$, the resistance, Rc, would be $Rc = \frac{rP \quad L}{A(1 - 0.01)} = \frac{rP \quad L}{A\,(0.99)}$. Thus the presence of a small number of chloroplastids would produce a small but significant change in resistance.

In condition B (fig. 3), $q = 0.5$, and the resistance, Rb, would be $Rb = \frac{rP \quad L}{A(1 - 0.5)} = \frac{rP \quad L}{A(0.5)}$. In condition C (fig. 3), $q = 0.9$, and here the resistance, Ra, would be $Ra = \frac{rP \quad L}{A(1 - 0.9)} = \frac{rP \quad L}{A(0.1)}$.

Thus:

$$\frac{Ra}{Rb} = \frac{rP \quad L}{A(1 - 0.9)} \cdot \frac{A(1 - 0.5)}{rP \quad L} = 5. \tag{6}$$

On the basis of these approximations (assuming the area, A, and the thickness, L, to be the same in all cœnocytes), Ra should be five times higher than Rb. Obviously this particular ratio need not be true in actual cases. However, dark-green cœnocytes were found to have a considerably higher resistance than pale-green cœnocytes as shown in table 2.

Twenty individual cœnocytes of *V. ventricosa* were collected from the same tide pool. These cœnocytes were all about the same size, 0.5 cm. in diameter, and were all spheroidal in shape. Ten of these cœnocytes were pale green and 10 were deep green in color. Considerable amounts of inter-chloroplastidal protoplasm separated the chloroplastids in the pale-green cœnocytes. A microsaltbridge was carefully inserted into the vacuole producing a type A puncture, and the resistance of each cœnocyte was measured. The measurements for each cœnocyte were made at 5 and 10 minutes after puncturing, and then the final measurement was recorded when the resistance remained constant.

TABLE 2—*Resistance and chloroplastid density.*

High chloroplastid density			Low chloroplastid density		
5 min.	10 min.	Const. Rp	5 min.	10 min.	Const. Rp
2000	2200	3500	450	850	1150
2600	2600	3600	750	1000	1500
2200	2600	3900	600	900	1650
2700	3000	4000	1200	1600	1800
1900	2400	4300	800	1200	1850
4200	4700	4800	800	1200	1900
2000	2600	4900	950	1150	1900
1700	2250	5000	1100	1650	1900
3800	4600	5150	800	1100	2000
2900	3500	5300	850	1600	2100
2600	3050	4450	900	1200	1800

Resistance values are given in ohms. Bottom line gives average Rp value for each group of measurements.

In 5 minutes the Ra/Rb ratio equaled 2.9; at 10 minutes Ra/Rb equaled 2.5; and the constant Ra/Rb ratio was 2.5. These ratios were calculated from the average resistance of each group of cœnocytes (see table 2). Ra and Rb represent the deep-green and the pale-green cœnocytes, respectively.

This evidence seems to indicate that the value, Ry, is a function of the density of chloroplastids. Then

$$Ry = \frac{rP \quad L}{Ay(1 - q)} \tag{7}$$

If equation (7) is substituted into equation (3), then

$$\frac{1}{Rp} = \frac{Ay(1 - q)}{rP \quad L} + \frac{Ax}{rX \quad L} \tag{8}$$

Occasionally in experiments which were carried over a prolonged period, there appeared to be a drop in resistance. It is quite possible that in these cases the chloroplastids contracted to a certain extent. This would tend to lower the value of q and, as may be seen in equation (7), this occurrence would bring about a decrease in Ry. It might be suggested here in passing that any agent affecting the orientation or contraction of chloroplastids, for example, light, may produce changes in other bioelectrical properties of the cœnocyte.

The protoplasm (designated by zone w) which has migrated toward the wall of the microsaltbridge consists mostly of hyaline protoplasm plus a few chloroplastids (fig. 1, C). Occasionally disintegrated chloroplastids and tiny vacuoles are present in this hyaline zone. Plowe (27) observed in *Griffithsia bornetiana* that plastids change their shape and position following injury by a micropipette. She also observed the swelling of nuclei and an increase in Brownian movement, but these phenomena were not observed in *V. ventricosa.*

While the cœnocyte is recovering from the puncture, we may assume that there are three general paths for the current, Ip, to flow (see fig. 1, B). A fraction, Ix, may flow through the cœnocyst (zone x); a fraction, Iw, may flow through the hyaline protoplasm (zone w) migrating toward the microsaltbridge; and a fraction, Iy, may flow through the uninjured portion of the protoplasmic layer (zone y). The resistance, Rp, based on the derivation of equation (1) would then be

$$\frac{1}{Rp} = \frac{Ay(1 - q)}{rP \quad L} + \frac{Ax}{rX \quad L} + \frac{1}{Rw} \tag{9}$$

At first Rw will have very little effect on Rp, and only when zone x becomes appreciably reduced does the effect of Rw enter. The area of zone w is a reciprocal of zone x. As the area of zone x decreases, the area of zone w increases (see fig. 1, B). The maximum area of zone w is always larger than the original area of zone x (also indicated in fig. 1, C). The boundary of the torn area is demarcated by a *ring* of chloroplastids. The area of this *ring* plus the area of zone x later make up the total area of zone w. For example, if zone x was 0.02 cm. in diameter, zone w may be as high as 0.05 cm. in diameter. However, the shapes of the two zones are similar. The changes in resistance occurring during the first few minutes are then largely a function of the changes in Rx.

After the protoplasm has migrated toward the microtip and obliterated zone x, most of the chloroplastids have condensed around the wall of the microtip (see fig. 1, C). This condensation of chloroplastids produces the ring of dark material as described by Osterhout, Damon, and Jacques (25).

These chloroplastids surrounding the microtip soon begin to migrate toward and gradually become redistributed within the hyaline zone w.

When the cœnocyte has completely recovered, this zone has the same microscopical appearance as any other part of the protoplasmic layer (see fig. 1, D). Recovery involves the redistribution of normal chloroplastids, the extrusion of disintegrated chloroplastids and the elimination of any tiny vacuoles that may have been present in zone w. Only those chloroplastids which are aggregated around the microtip actually migrate into zone w. No visible changes in position of chloroplastids in zone y were observed.

The movement of chloroplastids undoubtedly plays an important part in governing the changes in resistance following the closure of the gap in the protoplasmic layer. The resistance, Rw, may be expressed as follows:

$$Rw = \frac{rP^* \quad L^*}{Aw(1 - q)} \tag{10}$$

The specific resistance, rP*, of the hyaline protoplasm in zone w is considerably lower than the specific resistance, rP, of the protoplasm in zone y.[1] This difference is to be expected since the protoplasm in zone w contains some disintegrated chloroplastids and it appears to be quite alveolar at times. The thickness, L*, of zone w is also slightly less than the thickness, L, of the normal protoplasmic layer.

The fraction of current, Iw, flowing through zone w becomes lower as the chloroplastids migrate into this zone and become redistributed. This effect increases the quantity, q, which in turn lowers the value of area, Aw. With the redistribution of chloroplastids the quantity, Aw (1 — q), becomes less and with this Rw becomes higher. Along with the migration of chloroplastids, the disintegrated chloroplastid material is extruded from the protoplasm and the tiny vacuoles are also eliminated. These phenomena undoubtedly produce an increase in the value of rP*. The properties of zone w eventually become identical with those of the normal protoplasm,

[1] The area, Ay, of a cœnocyte 0.5 cm. in diameter is 0.78 cm.^{2} The area, Aw, of an average hyaline zone 0.05 cm. in diameter is nearly 0.002 cm.^{2} At 6 minutes Rp is approximately 50 per cent of the ultimate constant resistance (see table 1). At this time

$$1/Rx = 0, \text{ therefore } \frac{1}{Rw} = \frac{1}{Rp} - \frac{1}{Ry}$$

If Rp = 3100 ohms (at 6 minutes), and since Ry = constant Rp = 6300 ohms (from equation, 13), then Rw = 6100 ohms.

From equations (7) and (10), we may derive the following expression $\frac{rP}{rP^*} = \frac{Ry\,Ay(1 - q)\,L^*}{Rw\,Aw(1 - q)\,L}$. Let q in zone y = 0.9, and q in zone w = 0, then

$$\frac{rP}{rP^*} = \frac{6300 \times 0.78\ (1 - 0.9)\ L^*}{6100 \times 0.002\ (1 - 0)\ L}$$

$$= 40\frac{L^*}{L}$$

L* may range from 0.5L to 1.0L; the limits of rP/rP* will then range from 20 to 40. According to these approximations, rP at the time zone x is eliminated is 20 to 40 times higher than rP*.

and the value, rP*, approaches rP, also L* approaches L. When these changes occur, no further increase in Rp takes place.

If we substitute equation (10) in equation (9), then we may completely express the value of Rp at any time in an impaled *Valonia* cœnocyte (see fig. 1, B)

$$\frac{1}{Rp} = \frac{Ay(1-q)}{rP \quad L} + \frac{Ax}{rX \quad L} + \frac{Aw(1-q)}{rP^* \quad L^*} \tag{11}$$

Following the closure of the gap in the protoplasmic layer, Ax becomes zero (see fig. 1, C). Then as the chloroplastids become redistributed the effective area of zone w becomes reduced by the factor, $1 - q$ (see fig. 1, D). When the values of rP* and L* approach the values of rP and L, respectively, the resistance of the cœnocyte becomes

$$\frac{1}{Rp} = \frac{Ay(1-q) + Aw(1-q)}{rP \quad L} \tag{12}$$

But the value of $Aw(1-q)$ is very small in comparison to $Ay(1-q)$ and it may therefore be neglected. At complete recovery, Rp becomes

$$\frac{1}{Rp} = \frac{Ay(1-q)}{rP \quad L} = \frac{1}{Ry} \tag{13}$$

Therefore $Rp = Ry$.

Blinks (6) attributed the low resistance in impaled cœnocytes in part to profound changes in the entire protoplasmic surface. However, the electrical and microscopical evidence in this report suggests that these changes have occurred only in zone w and that the interior of the protoplasmic layer plays a very important part in these resistance changes.

In another series of cœnocytes, different rates and magnitudes of resistance changes may be measured. This is to be expected, because of the difficulty in exactly duplicating the puncture in various cœnocytes. Moreover it would be impossible to duplicate the cœnocytes. One variable may be eliminated by selecting cœnocytes according to their approximate chloroplastid density. The size is another factor which may be controlled.

However, in type B punctures, one may expect the following results. The initial Rp will be very low. After a short lag, depending largely on the size of the opening in the protoplasmic layer, there will be an increase in resistance. After the gap is closed another increase in resistance may be expected. The resistance should remain constant after the chloroplastids become redistributed and the disintegrated chloroplastids and other products of cytolysis are eliminated.

On the average, more time is needed for the chloroplastids to become redistributed than is necessary to close the gap in the protoplasmic layer. The changes in resistance, however, will parallel the morphological changes in the protoplasmic layer and these may be observed microscopically at all times.

COMPARISON OF TYPE A WITH TYPE B PUNCTURES

Cœnocytes in which a type A puncture was produced recovered rapidly and the constant resistance level was soon reached. The initial resistance in certain cases was 50 to 89 per cent of the constant resistance. A constant level in some cœnocytes was reached in 2 minutes. The time for complete recovery as indicated by the changes in resistance varied from 2 to 10

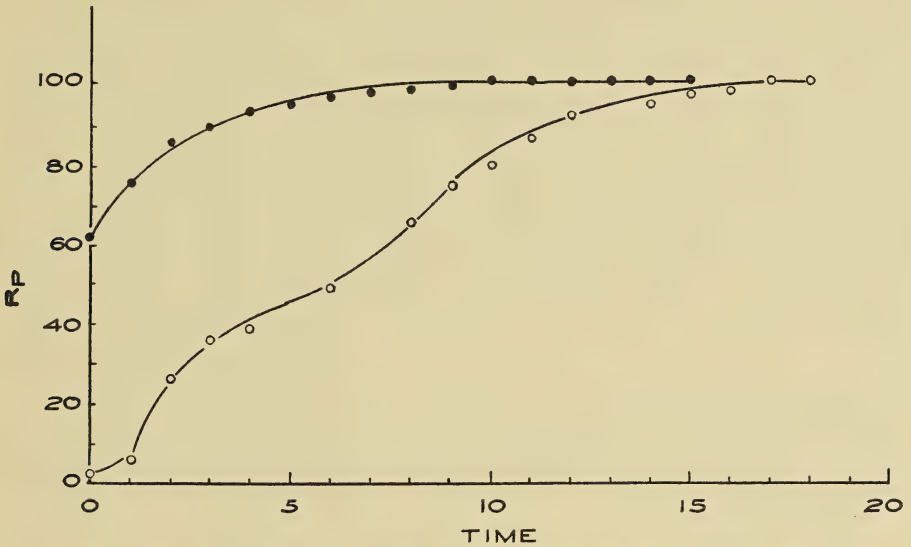

FIG. 4—Upper curve (solid black points) shows change in resistance with time in type A punctures (data from table 3). Lower curve indicates similar changes in type B punctures (data from table 1). The constant Rp was made equal to 100 and other values were calculated in percentage of the constant value. The time is given in minutes.

minutes. These data are indicated in table 3. A curve comparing the average type A resistance changes with the average type B changes is shown in figure 4. The constant resistance in each case was made equal to 100, and the other values were calculated in percentage of the constant resistance values.

In these cœnocytes there were no visible gaps in the protoplasmic layer, consequently zone x either did not exist or it was exceedingly small. The fraction of current that could flow through this region may therefore be neglected. Any changes in resistance were then due to the small zone corresponding to w in the type B punctures (similar to fig. 1, C). In a type A puncture the resistance, Rp, at any time is

$$\frac{1}{Rp} = \frac{Ay(1 - q)}{rP \quad L} + \frac{Aw(1 - q)}{rP^* \quad L^*} \tag{14}$$

The area of the hyaline zone, Aw, is very small, consequently Rw is initially high. There is much less disturbance of the protoplasm surrounding the microtip as evidenced by the very small amount of extruded material following recovery, thus rP* may be expected to be high at first.

Likewise the rate with which rP* approaches rP is evidently high. The rapid redistribution of chloroplastids produces a rapid increase in q. Thus

TABLE 3—*Type A Punctures*

Const. Rp	1400	950	1750	3700	1700	1600	1200	1400	Ave.
Time									
0	50	89	53	89	64	56	50	36	61
1	72	95	71	89	73	75	67	57	75
2	100	100	83	97	79	87	75	60	85
3	100	100	100	100	88	88	75	64	89
4	100	100	100	100	92	94	92	64	93
5	100	100	100	100	97	94	92	72	94
6	100	100	100	100	100	97	92	79	96
7	100	100	100	100	100	97	92	93	97
8	100	100	100	100	100	100	92	93	98
9	100	100	100	100	100	100	100	93	99
10	100	100	100	100	100	100	100	100	100
11	100	...	...	...	100	100	100	100	100
12	...	...	...	...	...	100	100	100	100
13	...	...	...	...	...	100	100	100	100
14	...	...	...	...	...	100	...	100	100
15	...	...	...	...	...	100	...	100	100

Upper line gives value of constant Rp in ohms. This value was made equal to 100 and the other values were calculated in percentage of the constant Rp. The rather low value for the constant resistance in this group of cœnocytes was due to their low initial chloroplastid content.

in a short time the quantity, $Aw(1 - q)$ approaches zero. At complete recovery rP* and L* are the same as rP and L, hence

$$\frac{1}{Rp} = \frac{Ay(1 - q) + Aw(1 - q)}{rP \quad L} \tag{15}$$

The value $Aw(1 - q)$ as in the type B punctures may be neglected, thus

$$\frac{1}{Rp} = \frac{Ay(1 - q)}{rP \quad L} = \frac{1}{Ry} \tag{16}$$

And $Rp = Ry$.

SUMMARY

(1) Glass microcapillaries, with tips ranging from 0.025 to 0.1 mm. in diameter and filled with vacuolar sap, served as microsaltbridges leading from the vacuole to a calomel half-cell. A larger glass tube (the macrosaltbridge), filled with sea-water, was used as a saltbridge leading from the sea-water surrounding the cœnocyte to another calomel half-cell. The *Valonia* cœnocytes were impaled on the tip of the microsaltbridge with the aid of a micromanipulator.

(2) The resistance of the chain: *microsaltbridge/ sea-water/ macrosaltbridge* is the basal resistance, Rb. The resistance of the chain: *microsaltbridge/ sap/ protoplasm/ sea-water/ macrosaltbridge* is the total re-

sistance, Rt. The resistance of the protoplasmic layer is designated by Rp, where Rp = Rt — Rb.

(3) The insertion of a microsaltbridge into the vacuole of a *Valonia* cœnocyte may produce one of three types of punctures. *Type A*: The microtip passes through the protoplasmic layer without tearing it. The protoplasm surrounding the microtip appears to be nearly normal as indicated by the very slight disturbance of chloroplastid distribution. *Type B*: There is a slight tearing reaction in the protoplasmic layer near the microtip leaving an opening 0.02 to 0.05 cm. in diameter. *Type C*: The tearing induced by a large microtip inserted by hand produces an opening in the protoplasmic layer from 0.1 to 0.2 cm. in diameter. Any injury greater than this is irreversible.

(4) It was found that cœnocytes with a high chloroplastid density have a constant Rp several times higher than cœnocytes with a low chloroplastid density. It is postulated that only the inter-chloroplastidal protoplasm is capable of conducting a current. An increase in chloroplastid density decreases the effective area of inter-chloroplastidal protoplasm, thereby producing an increase in Rp.

(5) The average initial Rp in type A punctures was 60 to 65 per cent of the constant Rp. In some cœnocytes a constant Rp was reached in a few minutes. This increase in Rp is caused largely by the redistribution of those chloroplastids around the microtip which were disturbed by the puncture.

(6) The average initial Rp in type B punctures was 2 to 3 per cent of the constant Rp. The constant Rp level was reached in an average time of 16 to 18 minutes. The closure of the torn region of the protoplasmic layer is responsible for the first increase in Rp. Later the redistribution of chloroplastids in the newly formed hyaline zone which filled the gap in the protoplasmic layer plus an increase in resistivity of the protoplasm in this zone cause a further increase in Rp. After the chloroplastids are redistributed in this hyaline zone, the disintegrated chloroplastids are extruded, and the tiny vacuoles are eliminated, no further increase in Rp takes place.

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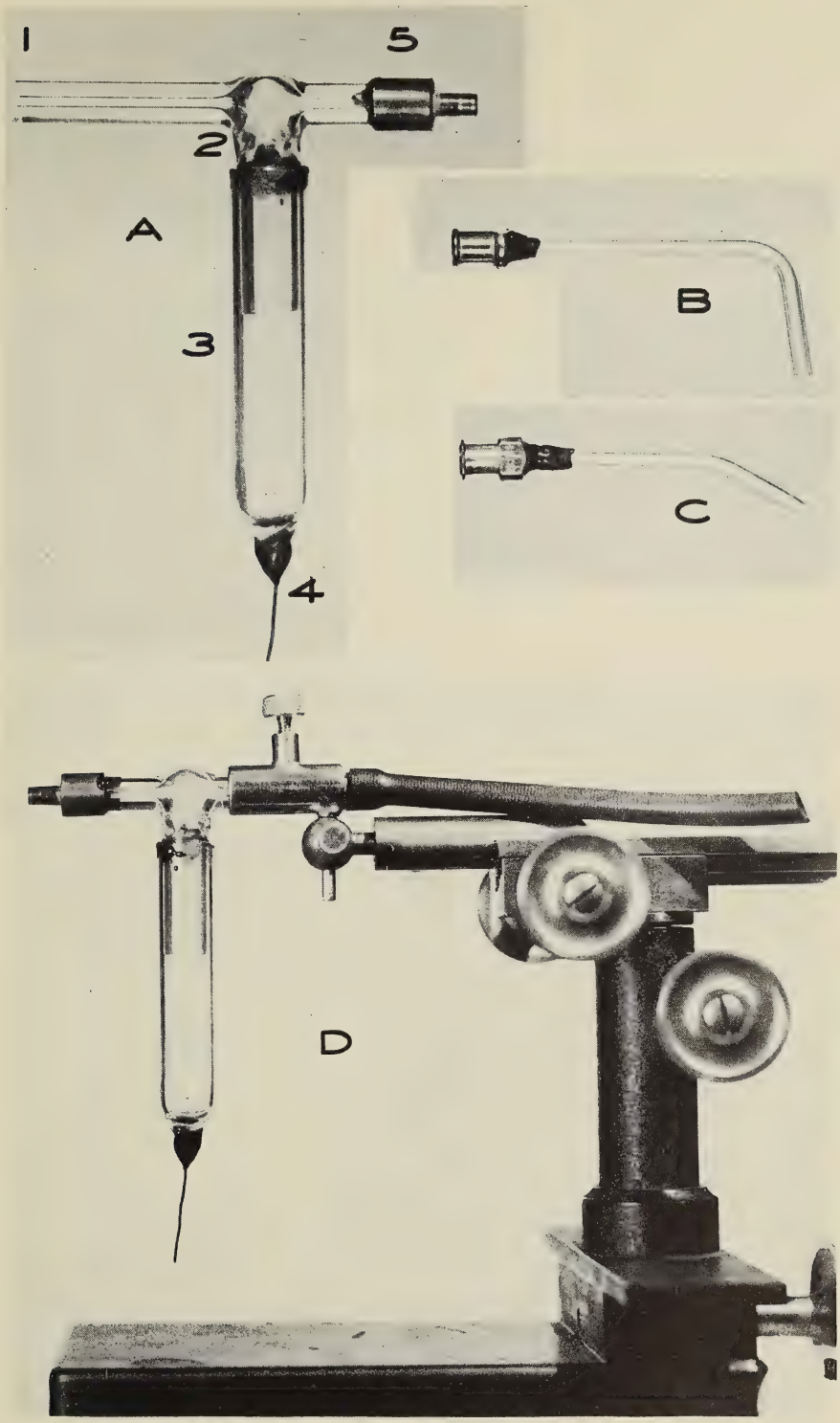

A. The electrode vessel. Capillary shank (1) is filled with vacuolar sap; stem (2) is filled with KCl-agar; tube (3) is filled with mercury, calomel, and saturated KCl; and a platinum wire (4) is sealed in end of tube. A Luer needle adaptor (5) is sealed to capillary shank.

B. The macrosaltbridge.

C. The microsaltbridge.

D. The electrode vessel and its holder are shown mounted on a Leitz Rack and pinion micromanipulator.

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